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This issue is dedicated to the memory of Professor **Bai Changjun** (13 October 1967 – 26 December 2018), Chinese pasture agronomist, who during his 28-yr career at the Chinese Academy of Tropical Agricultural Sciences (CATAS) and as Head of the Tropical Pasture Research Center at CATAS's Tropical Crops Genetic Resources Institute made outstanding contributions to the science of tropical pastures and forages.

Bai Changjun was an enthusiastic and most productive scientist in the area of collection, evaluation and utilization of tropical forage genetic resources; his colleagues in the research community will not forget his enthusiasm about *Stylosanthes*. He was a co-founder of *Tropical Grasslands-Forrajes Tropicales* and member of its Management Committee, and will be dearly missed by his friends and colleagues at the journal.

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Research Papers

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Research Paper

Long-term beef production from pastures established with and without annual crops compared with native savanna in the Eastern Plains of Colombia: A compilation and analysis of on-farm results 1979–2016

Producción de carne a largo plazo en pasturas establecidas con y sin cultivos anuales, en comparación con sabana nativa en los Llanos Orientales de Colombia: Recopilación y análisis de resultados obtenidos a nivel de finca durante 1979–2016

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Abstract

The Eastern Plains of Colombia ("Llanos") are made up of acid soils with low nutrient concentrations covered by tropical savannas, but they constitute a very important land resource for the country. The Llanos have traditionally supported extensive beef cattle production systems but are increasingly in demand for food, feed and oil production. The establishment of improved tropical pastures is a continuing trend as a means of intensifying beef production. Published data and the authors' own on-farm databases were collated and analyzed to estimate commercial cattle weight gains. A total of 198 records representing pastures directly drilled into native savanna and pastures undersown with rain-fed upland rice and maize were available for the 1979–2016 period, and were compared with savanna-based beef production. Records were available for sown paddocks 1–15 years in age that were managed solely by ranchers or by consensus between ranchers and researchers. Performance was not affected by who made the management decisions. Pastures sown in association with maize largely out-yielded all others, but observations on these paddocks were restricted to only 3 years and there was a steep fall in production with age. Differences between conventionally established and rice-associated pastures decreased rapidly over time, and production from these pastures did not differ over the longer term. Pasture age and stocking rate during the rainy season were the major factors influencing output from all systems. Overall, adjusted, long-term weight gains per year ranged between 11 and 386 kg/ha for savanna and maize undersown pastures. The data suggest a need to document long-term planned, regular, crop-pasture rotations, but none was available. The analyses provide valuable, long-term estimates of realized beef production under commercial conditions that should assist in decision-making by graziers and policy makers.

Keywords: Agropastoral systems; crop-livestock; extensive grazing; establishment methods; Llanos Orientales; tropical grasses.

Resumen

Los Llanos Orientales de Colombia se caracterizan por sabanas tropicales formadas sobre suelos ácidos, pobres en nutrientes, pero constituyen un recurso de tierras muy importante para el país. Su uso tradicional es la producción extensiva de ganado de carne pero hay una demanda creciente por la producción de ganado y cultivos, así como la

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explotación de petróleo. El reemplazo de las pasturas nativas de baja calidad por pasturas mejoradas es un proceso continuo para intensificar la producción ganadera. Se compilaron datos publicados, así como las bases de datos de los autores, para estimar las ganancias de peso vivo a nivel de fincas. Se dispuso de 198 registros que incluían potreros de sabana y de pasturas que se sembraron directamente o en asociación con maíz o arroz de secano. Los registros incluyeron casos de 1 a 15 años de duración. No se encontraron diferencias según las decisiones de manejo tomadas por el productor, o como resultado de discusión con investigadores. Pasturas establecidas bajo maíz superaron largamente la producción de las demás, pero los datos se refieren solo a tres años de registro y mostraron una caída pronunciada con la edad. Las diferencias iniciales entre pasturas convencionales y las establecidas con arroz desaparecieron en el largo plazo. Las ganancias anuales de peso a largo plazo y ajustadas estadísticamente variaron entre 11 y 386 kg por hectárea para sabana y el sistema maíz-pasto. La edad de las pasturas y la carga animal durante la estación lluviosa fueron los factores más importantes en determinar la producción de peso vivo. Se sugiere la necesidad de documentar la producción a largo plazo con base en rotaciones regulares y planeadas entre cultivos y pastos. Los resultados disponibles suministran estimados realistas de producción de carne a largo plazo en condiciones comerciales y deberían asistir en la toma de decisión por ganaderos y tomadores de decisiones políticas.

Palabras clave: Cultivos asociados; establecimiento de pasturas; pastoreo extensivo; pastos tropicales; sistemas agropastoriles.

Introduction

The well-drained savannas of Eastern Colombia cover 4.5 M ha and extend to the right of the Meta River until it discharges into the Orinoco River. Soils are Oxisols, typic Haplustox, containing kaolinitic clays with high concentrations of Al, low concentrations of P and cations, and low concentration and quality of organic matter (IGAC 1995). Deep soils are easily cultivated, but can seal and compact rapidly, diminishing water infiltration rates (Amézquita et al. 1998; Hoyos et al. 1999). The native savannas have traditionally been used for extensive cow-calf systems (Van Ausdal 2009), but production systems have evolved and diversified over the last few decades with the introduction of crops and sown pastures (Valencia and Leal 2004; Amézquita et al. 2013). Tropical grass pastures are generally established after removing the native vegetation but the development of adapted upland rice varieties, and later maize cultivars, allowed the undersowing of pastures with these cereals, thereby reducing establishment costs and taking advantage of residual crop fertilizer. While regular rotations of pastures with rice or maize were expected to develop economically viable and sustainable agropastoral systems (Sanz et al. 2004), producers have to change a culture dominated by extensive cattle ranching to more intensive crop-livestock systems. Various extension initiatives over the last 30 years gave rise to a variety of pastures established with one or more of the above systems, ranging from low external input, directly drilled pastures, to relatively high-input systems using rice or maize undersown with the same grass species or with more demanding species. These initiatives largely concentrated on the more levelled areas of the savannas ('Altillanura plana'; high savannas) and in a few cases, on valleys of the undulating, dissected savannas ('Serranía').

The primary objective of the current work was to compile, systematize and analyze the information available on beef production based on tropical sown ('improved') pastures established with different methods and compare it with production from native rangelands in the high savannas of the Colombian Eastern Plains. Secondly, we aimed to establish the magnitude of differences in beef output, their ranges and variability, and also to broadly assess the effects of soil and management on performance, if any. The underlying hypothesis was that long-term and practically relevant differences between systems would be found, and based on the authors' experience, differences in production between lighter and heavier soil textures were expected.

Materials and Methods

Location

The study region is located in the municipalities of Puerto López and Puerto Gaitán (3°55' and 4°20' N, 72°1' to 72°5' W; 120–220 masl), Meta Department, Colombia. Mean annual temperature is 26 °C with little variation throughout the year. Average annual rainfall is 2,649 and 2,158 mm for Puerto López and Puerto Gaitán, respectively, concentrated during April–November. Potential evapotranspiration amounts to 112 mm/month, and mean solar radiation is 4.47 kw-h/m^2 (HIMAT 1994).

Database

Data for control native savanna paddocks (Sav) and 3 contrasting and common pasture establishment methods (System), including direct drilling of pasture (Grass) after savanna, pasture undersown with an upland rice crop (Rgrass) and pasture undersown with a maize crop (Mgrass), were collected and contrasted. Adapted cultivars of both annual crops (Valencia and Leal 2004) were used in all cases. The systems varied in terms of land preparation practices and fertilizer application and are described by Sanz et al. (2004) and Rincón and Flórez (2013). In general, Grass systems implied seeding of the pasture species after savanna, with low levels of fertilizer application, including 20 kg P, 20 kg K, 100 kg Ca, 20 kg Mg and 10 kg S/ha; Rgrass systems consisted of the joint planting of upland rice and pasture species following recommended practices (Sanz et al. 2004), including dolomitic limestone (90 kg Ca and 27 kg Mg/ha), 50 kg P, 100 kg K and 5 kg Zn/ha; and the Mgrass system involved undersowing pasture species with a maize crop with much higher fertilizer inputs. The data for 4 farms reported by Rincón and Flórez (2013) represented maize-pasture combinations that received dolomitic lime (550 kg Ca and 225 kg Mg/ha), 1,000 kg rock phosphate and 400 kg gypsum/ha; the maize crop received in addition 128 kg N and 120 kg P (40 kg soluble, 80 kg as rock phosphate)/ha. An additional farm (Hoyos and Vera, unpublished) grew drilled maize undersown with *B. decumbens* fertilized with dolomitic limestone (330 kg Ca and 135 kg Mg), 80 kg N, 50 kg P, 100 kg K and 20 kg Zn/ha.

Sown grass species included *Andropogon gayanus* (Ag, cv. Carimagua 1) and commercial *Brachiaria humidicola* (Bh, CIAT 679) for the Grass system; *Brachiaria dictyoneura* (Bdic, cv. Llanero; reclassified as *B. humidicola*, Cook and Schultze-Kraft 2015) in the Grass and Rgrass systems, and hybrid *Brachiaria* cv. Mulato II and *Brachiaria brizantha* cv. Toledo in the Mgrass system. These grass species were generally associated with a variety of legumes that over the long term made a low contribution to botanical composition and are not further considered here. Initial analyses indicated that differences between systems were larger than between grass species within systems, and the present paper focusses on the former, independently of the grass species sown.

A database of steer liveweight gains in relation to System was compiled and included published peer-reviewed papers, institutional bulletins and conference proceedings between the years 1979 and 2016, but 70% of the data originated from a large number of unpublished on-farm recordings made by the authors. The total number of individual records was 198 for 17 farms and paddock sizes varied between 100 and 250 ha in the case of savannas, and between 10 and 80 ha for sown species; number of animals per paddock ranged between 15 and 90 head per year. In all cases, animals were replaced annually. Not all animals were present throughout a year in the respective paddocks given changes brought about by sales, purchases and other management decisions taken by graziers, so that weighted averages were used when necessary.

Relatively small, intensively-managed, grazing plot experiments run at the regional Research Center Carimagua (CORPOICA) were not included, and served as controls for the subsequent discussion. The number and types of records available are summarized in Table 3. The final version of the database was thoroughly checked for consistency and errors.

Definition of variables and production systems

The following variables were recorded: year sown; year observed; pasture age (years); soil texture; soil apparent density (apdensity); species and cultivars sown; stocking rates during wet and dry seasons (SRw and SRd, respectively, in head/ha); daily liveweight gains during the wet and dry seasons (DLWGw and DLWGd in g/head); liveweight gains during the respective seasons (LWGw and LWGd in kg/head); yearly liveweight gain (LWGhayr in kg/ha); and production system (System). Live weights of grazing beef cattle (yearlings and steers) were recorded 3 or 4 times during the wet season and at least twice in the dry season, to allow calculation of daily liveweight changes in both seasons. A further dummy variable, 'dummymgmt', was defined based on the decision-making process regarding establishment and management of the system such that a value of -1 was assigned to researcher-led processes, 0 to joint farmer-researcher management decisions and +1 to solely farmer decision making. Soil samples were generally taken at the time of planting and at 0–20 cm depth. More detailed data on soil texture and apparent density were evaluated on the same ranches as well as on others, and are discussed at length by Hoyos et al. (1999) and Rivas et al. (2004).

For simplicity of presentation, and in view of large between-farms variability, subsequent analyses grouped wet season stocking rates into 4 discrete categories (very low = <0.5; low = 0.5 to <1.50; medium = 1.5 to <3.0; and high = ≥3 animals/ha). Similarly, pasture age was grouped into 3 categories (low = 2 or less; medium = >2 and <6; and high = 6 years and greater).

Lengths of wet and dry seasons were standardized to 210 and 155 days, respectively, based on a large volume of existing climate data for the Altillanura (Hoyos et al. 1999). Available data on beef production referred to pastures ranging in age between 1 and 15 years, but not every age was available for every paddock such that grazing data in individual paddocks extended to a maximum of 7 years. A small number of records did not include dry season performance, most frequently because pastures were rarely used in that season or due to other management issues.

Statistical analyses

All statistical analyses were carried out with SAS/STAT 9.2 (SAS 2009).

Initial analyses involved the calculation of descriptive statistics for the available quantitative variables (Table 2), verification of the distributional properties of the data and extensive graphical analyses. Canonical discriminant analyses (proc CANDISC) were carried out to establish orthogonal canonical variables that best discriminate between production systems. The primary goal of the discriminant analyses was to "find the dimension or dimensions along which groups differ, and to find classificatory functions to predict group membership" (Tabachnick and Fidell 2007).

Factors selected for the analyses included the following: dummymgmt, apdensity, year observed, DLWGw, DLWGd, SRw, SRd, LWGhaw and LWGhad. The number of canonical variables selected was based on their significance, but was restricted to a maximum of 3, and Mahalanobis square distances were assessed to determine distances between production systems. Preliminary analyses of variance and stepwise linear regressions were also used to select categorical and quantitative variables used in the final analyses.

Definitive analyses were based on fitting generalized linear mixed models (GLMM) using proc GLIMMIX (Gbur et al. 2012) to data, including the following factors: farm as random variable; System as categorical variable; SRw (or discrete stocking rates as previously described), pastureage (or discrete age), soil organic matter, clay and sand %, and soil P, Ca, Mg and K concentrations as quantitative variables; and lastly the interactions of pastureage × SRw and pastureage × System. Numerous other interactions were tested and discarded due to very low probability values. The final GLMM model included the following factors: farm as random variable, System, SRw, pastureage × System and pastureage × SRw, since these variables were significant, but when comparing treatments, preference was given to standard errors of the mean (s.e.m.) and confidence intervals (CI) in view of current discussions on the value and interpretation of P values (Wasserstein and Lazar 2016).

Results

Soils

Soils supporting the systems studied are described in Table 1.

Statistics of the database

Descriptive statistics for the available farm data are shown in Table 2, while Table 3 groups statistics based on the previously described System.

Canonical discrimination

Canonical analyses identified 3 distinct canonical variables that were partially successful in separating Systems from one another, when restricted to observations made during the wet season and during the whole year. The results of these analyses guided subsequent analyses of variance. The first 2 canonical variables (Figure 1) accounted for 97.1 and 97.8% of the variance ($P<0.0001$) for all multivariate statistical tests (e.g. Wilks' Lambda, Pillai's trace) for analyses which referred to the wet season and the whole year, respectively. In both cases, the first canonical variable, which accounted for 75.5 and 75.3% of the variance, respectively ($P<0.0001$), was dominated by the effects of SRw, SRd and pastureage, indicating the major influence of these variables on performance of the various systems.

Table 1. Soil texture and chemical composition (0–20 cm) across a range of farms and pastures. SOM = soil organic matter.

	Sand (%)	Clay (%)	SOM (%)	pH	P (ppm)	Al (cmol/kg)	Ca (cmol/kg)	Mg (cmol/kg)	K (cmol/kg)
N	48	48	54	54	56	54	54	54	54
Mean	42.2	33.4	3.1	4.5	3.6	2.0	0.19	0.07	0.07
SD	21.47	13.43	1.5	0.4	2.5	1.1	0.08	0.04	0.02
Min	12.0	15.4	1.1	3.4	1.3	0.2	0.06	0.02	0.03
Max	77.3	54.1	6.0	5.2	16.2	3.8	0.36	0.16	0.14

Table 2. Descriptive statistics of variables monitored in farm paddocks: DLWG = daily liveweight gain during wet and dry seasons; SR = stocking rate during the specified season; LWG = liveweight gain/head during the specified season or year; and LWGha = liveweight gain/ha during the season or per year.

Variable	N	Mean	Median	Min	Max	s.e.m.
ApDensity	54	1.39	1.27	1.15	1.7	0.01
Pastureage (yr)	181	3.94	3	1	15	0.24
DLWGw (g/hd)	197	489	501	84	947	13.5
SRw (hd/ha)	197	1.72	1.7	0.12	5.09	0.07
LWGw (kg/hd)	197	102	105	18	199	2.9
LWGhaw (kg/ha)	197	188	165	3	673	9.8
DLWGd (g/hd)	153	186	197	-268	795	17.5
SRd (hd/ha)	152	1.11	0.99	0	6.3	0.07
LWGd (kg/hd)	152	29	30	-42	123	2.7
LWGha (kg/ha)	151	31	22	-95	273	4.1
LWGyr (kg/hd)	152	130	122	22	276	5
SRyr (hd/ha)	145	1.72	1.58	0.12	4.09	0.06
LWGhayr (kg/ha)	156	231	200	5	701	13

Table 3. Descriptive statistics of main variables monitored in farm paddocks according to system of pasture establishment (System): Grass = conventional drilling of pasture after savanna; Rgrass = pasture undersown with rainfed rice crop; Mgrass = pasture undersown with maize crop; and Sav = native savanna. N = number of observations over the duration of the recording process; DLWG = daily liveweight gain during the wet or dry season; SR = stocking rate during the specified season or whole year; LWGhayr = liveweight gain/ha during the year.

System	N	Variable	Mean	Median	Min	Max	s.e.m.
Grass	132	DLWGw (g/hd)	499	515	84	894	14.8
		SRw (hd/ha)	1.60	1.60	0.27	3.00	0.04
		DLWGd (g/hd)	146	140	-268	782	23.2
		SRd (hd/ha)	1.27	1.09	0.00	3.63	0.08
		SRyr (hd/ha)	1.59	1.58	0.77	3.00	0.05
		LWGhayr (kg/yr)	191	172	45	476	10.0
Rgrass	20	DLWGw (g/hd)	441	437	107	774	43.4
		SRw (hd/ha)	2.02	1.81	1.09	4.08	0.16
		DLWGd (g/hd)	339	313	-4	795	46.5
		SRd (hd/ha)	1.34	1.12	0.75	2.38	0.12
		SRyr (hd/ha)	1.77	1.51	1.02	4.09	0.15
		LWGhayr (kg/yr)	257	240	60	587	29.4
Mgrass	28	DLWGw (g/hd)	654	665	269	947	26.7
		SRw (hd/ha)	3.07	3.01	1.17	5.09	0.18
		DLWGd (g/hd)	297	274	205	721	20.3
		SRd (hd/ha)	0.87	0.55	0.46	6.30	0.22
		SRyr (hd/ha)	2.53	2.51	1.52	3.99	0.12
		LWGhayr (kg/yr)	464	459	209	700	26.4
Sav	18	DLWGw (g/hd)	234	237	117	391	20.4
		SRw (hd/ha)	0.23	0.20	0.12	0.50	0.03
		DLWGd (g/hd)	21	84	-252	250	38.1
		SRd (hd/ha)	0.22	0.14	0.12	0.50	0.04
		SRyr (hd/ha)	0.14	0.13	0.12	0.23	0.01
		LWGhayr (kg/yr)	11	11	5	23	1.4

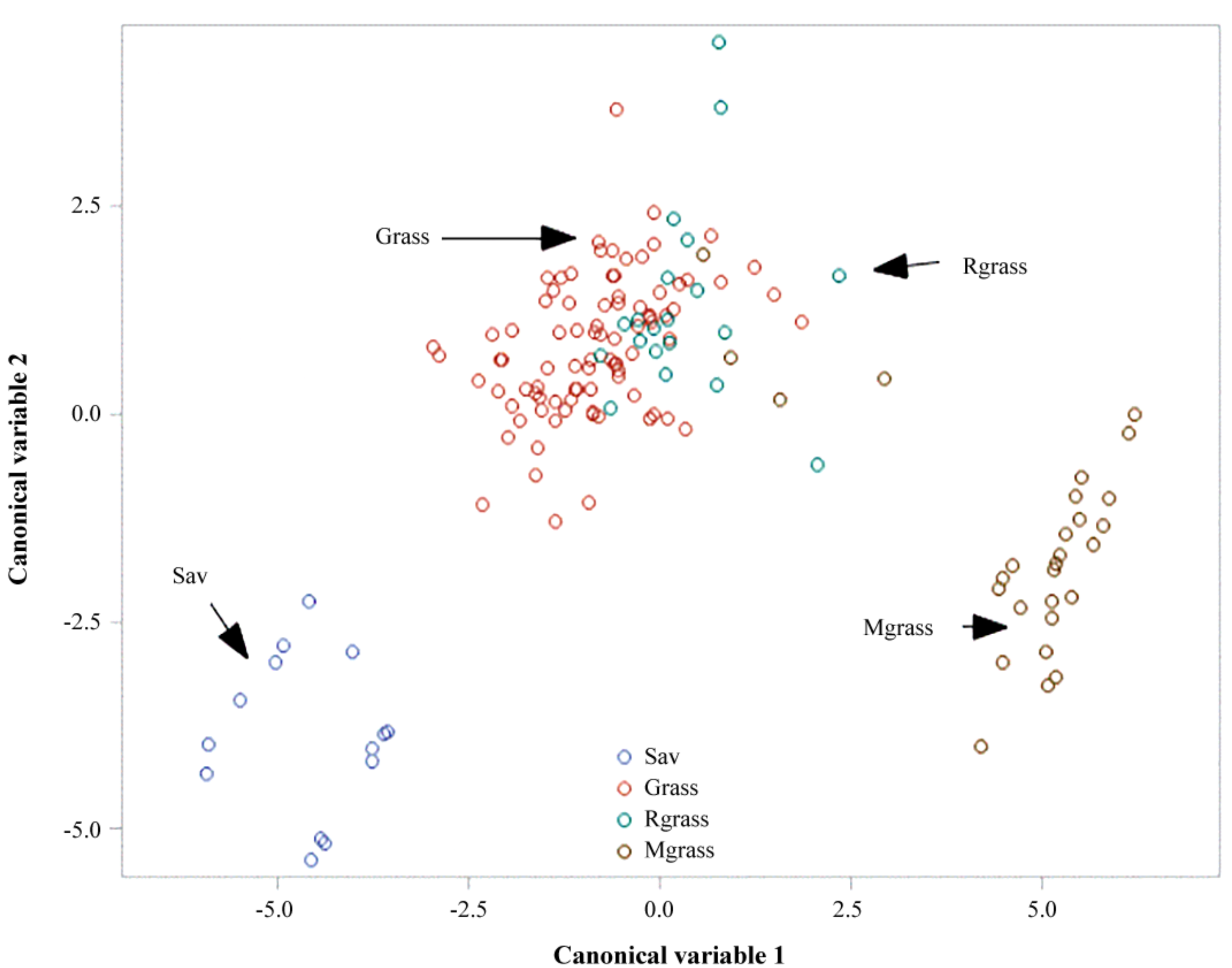

Figure 1. Plot of the first 2 canonical variables showing the separation between production systems achieved by canonical discrimination. Further details in text.

These 2 canonical variables successfully discriminated between the 2 extreme Systems, namely Sav and Mgrass, as shown in Figure 1, whereas the remaining 2 systems overlapped, making further separation impossible. In fact, the Mahalanobis square distances between Grass and Rgrass were very small, although significant, for both analyses.

The loadings or canonical structures equivalent to the correlations between the original and the canonical variables for pastureage, SRw, LWGhaw and DLWGw were 0.92, 0.81, 0.78 and 0.56, respectively, for the first canonical variable, indicating the importance of the original variables in determining the structure of the database, whereas soil texture and apparent density had very small correlations.

Influential effects

On-farm grazing management of sown pastures consisted of some measure of rotational grazing, varying in the length of the grazing/spelling periods, whereas savanna paddocks were generally subject to continuous grazing.

Animal performance was largely determined by performance during the wet season so further analyses of the database focused mainly on that season. Initial analysis with a full model including all experimental factors showed that, contrary to the initial hypothesis of the authors, soil texture (P = 0.39), soil apparent density (P = 0.29) and dummymgmt (P = 0.48) did not influence liveweight gain/hd during the wet season. A similar non-significant result was observed for liveweight gain/ha during the wet season, so these 3 variables were excluded from subsequent analyses. On the other hand, pasture age, stocking rate during the wet season, and several interactions among them consistently had significant effects (P values at least <0.01 in all cases) as discussed below.

Animal performance

Mean (Least square means, LSMEANS) long-term weight gains for the 3 sown pasture systems (System) are shown in Table 4. Gains on Grass and Rgrass systems were similar (P>0.05) during the wet season or whole year, and were significantly (P<0.01) lower than those on Mgrass, the differences being of practical relevance. The performance of Rgrass during the dry season following the rice harvest was unexpectedly higher than that of the other 2 systems and this effect carried over into longer-term analyses.

Table 4. Long-term mean liveweight gains (kg/ha) per season and per year for 3 systems of pasture establishment (System): Grass = conventional drilling of pasture after savanna; Rgrass = pasture undersown with rainfed rice crop; Mgrass = pasture undersown with maize crop. Data consist of least square means (LSMEANS) followed by the respective standard errors and confidence intervals (P = 0.05; in parentheses).

System	Wet season	Dry season	Year
Grass	207 ± 17	25 ± 4	234 ± 17
	(182–232)	(7–43)	(197–272)
Rgrass	171 ± 19	68 ±11	253 ± 25
	(131–209)	(45–91)	(202–304)
Mgrass	270 ± 20	48 ± 12	333 ± 26
	(229–310)	(23–73)	(281–386)

Throughout all analyses, the effects of stocking rate and pasture age were very consistent and significant (P<0.0001). Wide dispersion and variability of the above parameters were present in the database and for purposes of summarizing, ages of pastures and stocking rates were grouped into discrete categories as previously described. The most relevant and significant LSMEANS for the respective parameters are summarized in Tables 5 and 6. The breakdown of these 2 tables indicates clearly the relative abundance of observations for the various combinations of systems and variables, some of which are absent or not plausible, and where the number of observations is low, the LSMEANS should be viewed only as indicative of possible trends.

Table 5. Effects of stocking rate (head/ha) on animal performance from 4 contrasting systems: Grass = conventional drilling of pasture after savanna; Rgrass = pasture undersown with rainfed rice crop; Mgrass = pasture undersown with maize crop; and Sav = native savanna. Data consist of least square means (LSMEANS) ± standard errors where appropriate. Empty table cells indicate data not available. N = number of records; SRw = stocking rate during the wet season; DLWGw = daily liveweight gain during the wet season; and LWGha = liveweight gain/ha/year.

System	Parameter	Stocking rate (hd/ha)		
		Low	Medium	High
Grass	N	53	67	3
	SRw (hd/ha)	1.25	1.92	3.00
	SRyr (hd/ha)	1.18	1.80	3.00
	DLWGw (g/hd)	535 ± 21	474 ± 22	400 ± 10
	LWGha (kg/ha)	147 ± 11	215 ± 13	323 ± 14
Rgrass	N	3	13	2[1]
	SRw (hd/ha)	1.36	1.83	3.58
	SRyr (hd/ha)	1.39	1.18[2]	2.80
	DLWGw (g/hd)	571 ± 84	407 ± 51	318 ± 171
	LWGha (kg/ha)	248 ± 26	212 ± 26	288 ± 130
Mgrass	N		10	14
	SRw (hd/ha)		2.53	3.83
	SRyr (hd/ha)		2.08	3.05
	DLWGw (g/hd)		660 ± 37	686 ± 35
	LWGha (kg/ha)		366 ± 26	560 ± 27
Sav	N	18		
	SRw (hd/ha)	0.23		
	SRyr (hd/ha)	0.22		
	DLWGw (g/hd)	238 ± 20		
	LWGha (kg/ha)	11 ± 1.4		

[1]Caution is required in interpreting the values in this section.
[2]The low annual SR compared with that of the wet season was due to pastures left ungrazed during the dry season.

The rate of change of DLWGw with increasing age of pasture was significantly steeper (P<0.01) in Mgrass than in the other 2 systems that did not differ from each other. Similar regressions of DLWGw on stocking rate yielded parallel lines between the 3 systems (not shown), with a significant difference between Mgrass and the other 2 systems. The magnitudes of these differences were of practical relevance and are illustrated in Tables 5 and 6. Within years and over the long term, dry season performance did not differ between systems (P>0.05) and there was no influence of any of the experimental factors.

As indicated above, there were significant (P<0.001) interactions between System and discrete age, as well as between System and discrete SRw. Weight gains per ha

Table 6. Effects of pasture age (years) on animal performance from 4 contrasting systems: Grass = conventional drilling of pasture after savanna; Rgrass = pasture undersown with rainfed rice crop; Mgrass = pasture undersown with maize crop; Sav = native savanna. Data consist of least square means (LSMEANS) ± standard errors where appropriate. Empty table cells indicate data not available. N = number of observations; SRyr = stocking rate during the whole year; DLWGw = daily liveweight gain during wet season; and LWGhayr = liveweight gain/ha during the year.

System	Parameter	Pasture age (yr)		
		Low	Medium	High
Grass	N	41	69	18
	Pasture age (yr)	1.59	3.93	6.83
	SRyr (hd/ha)	1.50	1.75	1.46
	DLWGw (g/hd)	562 ± 23	471 ± 22	439 ± 35
	LWGhayr (kg/ha)	239 ± 21	189 ± 13	137 ± 11
Rgrass	N	8	8	2[1]
	Pasture age (yr)	1.50	3.63	7.00
	SRyr (hd/ha)	2.07	1.40	1.41
	DLWGw (g/hd)	448 ± 61	346 ± 64	644 ± 70
	LWGhayr (kg/ha)	273 ± 30	169 ± 33	270 ± 24
Mgrass	N	16	8	
	Pasture age (yr)	1.50	3.00	
	SRyr (hd/ha)	2.84	2.28	
	DLWGw (g/hd)	714 ± 28	598 ± 40	
	LWGhayr (kg/ha)	548 ± 29	360 ± 29	
Sav	N			17
	Pasture age (yr)			Unknown
	SRyr (hd/ha)			0.12
	DLWGw (g/hd)			244 ± 21
	LWGhayr (kg/ha)			11 ± 1.5

[1]Caution is required in interpreting the values in this section.

and per year increased steeply in Mgrass (Table 5) with increase in stocking rate and significantly less so in Grass and Rgrass. On the other hand, the negative effect of age on annual output per ha was significantly more pronounced in Mgrass than in the other 2 systems.

Discussion

Until a few decades ago, the Eastern Plains of Colombia were considered marginal lands (Rausch 2013) but discovery of valuable oil resources, development of appropriate agricultural technologies, ingress of large investors and continued increase in demand for land and food production have made the region a significant link in valuable food, feed and oil production chains. Furthermore, lands previously regarded as fit only for very extensive cattle systems are increasingly in demand for new crops, conservation of land and biodiversity and as reserves for original peoples and cultures (Navas-Ríos 1996; Boron et al. 2016), but beef cattle production still constitutes the main land use system of the region.

Information regarding weight gains by unsupplemented stockers and steers grazing tropical pasture species in the savannas of Eastern Colombia is available from the mid-1970s until the present time and is widely dispersed among a few peer-reviewed journal papers, numerous institutional publications of varying circulation and unpublished databases. Pasture species have been established using 3 main seeding methods, including: conventional drilling after removing native savanna species along with a low level of P fertilizer occasionally complemented with Ca and K; undersown with upland rice with recommended soil preparation methods and application of fertilizers (Sanz et al. 2004); and undersown with maize using a more complex system of land preparation and higher external inputs (Rincón and Flórez 2013). With regard to soil nutrient status, Hoyos et al. (1992a) conducted an extensive and detailed survey of on-ranch soil properties and reported large variations in soil texture, and strong and significant relationships between soil texture and nutrient concentrations, factors that would contribute to increased variability in any comparison between sites.

Soils supporting these pastures are generally nutrient-deficient and deep, and originally had good but very fragile structure, as revealed by previous surveys and modelling efforts (Hoyos et al. 1992a; Brito 1995). While soil texture is extremely variable, in very general terms, as one moves towards the east of the region, soils become sandier. Contrary to our initial hypothesis, soil texture had only a limited influence on beef production per head and per hectare. It could be argued that this is an artifact brought about by the high between-farms and within-systems variability observed, and by other confounding factors already noted. Very few systematic observations are available on differences in animal output associated with variations in soil texture. However, Rivera (1985) used a designed, on-station experiment to report large and significant differences in wet season weight gains of heifers grazing savannas on light soils (sand 52.3%, clay 25.7%, 1.3 % SOM; 140±23 g/d) and savannas on heavier soils (19.8% sand, 40.2% clay, 2.9% SOM; 309±51 g/d). As well as having different soils these savannas varied somewhat in botanical composition, which might have influenced the outcome. When comparing systems over extended areas, these and other difficulties and confounding factors have been highlighted in Australian (Winter et al. 1991; Bortolussi et al. 2005) and Uruguayan (Picasso et al. 2014) studies that aimed to establish baselines for pasture management, beef production and quality, and sustainable development across large and varied rangeland areas.

Contrary to previous observations regarding the frequent use of reclamation methods on degraded *Brachiaria decumbens* pastures reported by Vera et al. (1998), the current database, which contains information for just a single *B. decumbens* paddock, did not contain any instances of pastures renovated by any means. With regard to pasture management, some form of alternative grazing was common, which is broadly comparable with the results of a previous, independent survey of pastures managed exclusively by ranchers, that found relatively long periods of occupation were prevalent, but these alternated with rests of 73 d during the wet season (Hoyos et al. 1992b).

The modest variation in DLWGw with age in Grass and Rgrass was unexpected, as previous studies indicated in some cases a pronounced decrease in output with increasing age of pastures. Lascano and Euclides (1996) reported a marked decrease in performance with age in an experimental *Brachiaria humidicola* pasture located in Carimagua. On the other hand, using large paddocks, Ramírez-Restrepo and Vera (2018) reported limited variation between years in an equally old pasture of *B. humidicola* located only 1 km away from the long-term experiments summarized by Lascano and Euclides (1996). These contrasting results explain in part the large variation between farms and years found in our database, all of which contributed to relatively large random and experimental residuals. Not-withstanding these constraints, the present data and analyses provide a broad prediction of realized, commercial, beef cattle weight gains for a large and diverse area over a significant number of years, given extant management styles and physical inputs. The confidence intervals offer an adequate statistical range of weight gains, and given the variability previously noted, the intervals are relatively wide but nevertheless clearly differentiate between the extreme systems tested. Nevertheless, the comparison between Mgrass and the other systems is constrained by data being limited to only 3 years of testing. In this context, a serious limitation of our results is the absence of data on truly integrated crop-livestock systems whereby there is a regular and planned rotation of crops and livestock (e.g. Landers 2007), since our database contains no examples of animal performance on pasture-crop rotations.

Within the limits of the available data, the calculated large interactions between systems, stocking rates and pasture longevity (pasture age prior to renovation, if any) show the major trade-offs involved in these systems that challenge temporal decision making and resultant financial outcomes, issues that should be best dealt with via simulation modeling (e.g. Finlayson et al. 2012).

Existing highly controlled, research station-based experimentation comparing the crop-pasture systems under study (CIAT 1992) is limited (reviewed by Sanz et al. 2004) and would assign a high prior probability (in a Bayesian sense) to the existence of significant and practically meaningful differences between them. For example, over a period of 482 d following the rice harvest, undersown pastures of *Andropogon gayanus-Stylosanthes capitata* and *Brachiaria dictyoneura-Centrosema acutifolium* yielded weight gains of 705 and 629 g/d with stocking rates of 1.78 and 1.71 animals/ha, respectively, much higher than that possible with the Grass systems recorded in our results. The agronomic, animal and economic performance of the Rgrass system resemble results obtained in a wide range of conditions in the Brazilian Cerrados (e.g. Kluthcouski et al. 2004). Current results based on uncontrolled environmental conditions showed very large variation, only part of which could be assigned to between-farm differences. The observed variation masked differences, particularly when long-term comparisons between the Grass and Rgrass systems were carried out, which suggests that the initial advantage of Rgrass in terms of animal output over the Grass system is lost over periods >4 years. On the other hand, short-term results were available for the Mgrass system that showed large and significant differences in terms of animal outputs relative to the other 2 systems during the initial 3 years. Longer-term trends for Mgrass are not available, but it is reasonable to hypothesize that much would depend on the levels of maintenance fertilizer applied subsequently, if any. Clearly, long-term research is required on this system to determine how and when it reaches a steady state, and its relationship with maintenance fertilizer management, though maintenance fertilizer requirements of *Brachiaria* species appear to be modest (Rao et al. 1996; Rincón 1999). On-station grazing studies of *B. decumbens* tend to support this view (Lascano and Euclides 1996). In the absence of this research, present results argue for the establishment of planned, regular rotations of pastures with annual crops to maintain production. Nevertheless, adoption of crop-pasture rotations has been low (González et al. 2017). Other pasture establishment methods are under investigation, such as in silvopastoral systems, but at the time of writing there are no peer-reviewed, published long-term data for the region, despite a strong institutional interest (Uribe et al. 2011; Rincón and Flórez 2013) in the system.

Another issue that arises from the analysis of our database and its comparison with controlled, on-station grazing studies is the problem of extrapolation of data from small, uniform and intensively managed experimental grazing plots to large on-farm paddocks and, related to this, the existence of yield gaps, namely, the eventual difference between potential yield measured in small plots and that achieved in commercial practice. Differences between experiment station-based, small-plot grazing trials and larger on-farm paddocks have been recorded in some, but not all cases (e.g. Elliott 1966; Southcott et al. 1968; Scanlan et al. 2013) for a number of reasons. While yield gaps are better documented in crops (Beddow et al. 2014), early work by Davidson and Martin (1968) extended the concept to animal production. In the context of the present data it would be difficult to argue about the existence of consistent, repeatable differences between on-station and on-ranch animal performance, but one should examine the whole crop-cattle system to assess yield gaps and physical and economic sustainability (van der Linden et al. 2018), an exercise that remains to be carried out. Given the soil nutrient constraints already noted, the results observed in the first few years of the crop-pasture system, particularly that of Mgrass, and the subsequent decrease of outputs with time, are clear indications that yields of pastures, as reflected by weight gains per hectare, are severely limited by soil resources. To the authors' knowledge, there are no published data on potential pasture yields in the absence of these limitations, so as to evaluate the true nature and size of the potential gap. Furthermore, and in terms of animal performance, animals with higher genetic potential than those currently available would be required to express the full potential of those improved systems, since there is the strong possibility of an environment-genotype interaction (Euclides et al. 2014).

The long-term consequences of various establishment and management methods in terms of subsequent beef production involve a series of confounded effects, possibly including different grass species. As noted previously, differences between systems were larger than between pastures species. Nevertheless, these differences between systems, species and even subsequent pasture management practices are implicit to the respective production systems. The current data and results allow broad generalizations, and the authors consider that the number of records and duration of the observations plus some of the very significant practical differences reported here constitute a valuable resource that may determine future R&D initiatives, and may enlighten future policy decision making.

The establishment techniques and production systems documented here have been tested on-station and on-ranch and have proved to be productive and economic (Rivas et al. 2004; Rincón and Flórez 2013). Nevertheless, challenges remain in terms of their integration with the rest of the property's resources and the required decision making processes in the context of developing sustainable land uses (Boron et al. 2016). For example, it is hypothesized that grazing management, including seasonal adjustments in stocking rates in systems such as Mgrass, may present a challenge for livestock owners accustomed to less-demanding, more-extensive systems.

Furthermore, as the cost of production increases, effectively matching animal types to pasture and feed resources becomes more important (Mulliniks et al. 2015), an issue that has received scant systematic attention in the region to date. Ramírez-Restrepo and Vera (2018) showed large and significant differences in performance between animal categories and genders grazing 2 very different grass species, a situation further compounded by small, but significant differences in predicted methane output, which suggests again a need to consider whole-system performance (van der Linden et al. 2018) across a range of production scenarios.

As Winter et al. (1991) indicated many years ago, much depends on individual owners and, we add, an enabling policy environment.

Conclusions

The results showed a clear, significant and practically important difference in liveweight gain between the native savanna stocked at 0.2 head/ha and improved pastures either sown directly after savanna or undersown with rice and maize stocked at 1 or more animals per ha.

While the relatively highly fertilized maize-pasture combination out-yielded all other systems, data were limited to the first 3 years after sowing, and weight gains per ha declined steeply over that time interval.

The rice-pasture system tended to out-yield conventionally planted pastures during the first 2–3 years but mean differences over the longer term disappeared.

There was no evidence that soil texture affected the outcomes in terms of animal performance, and management decisions taken by farmers as opposed to those agreed upon with researchers did not influence animal performance.

Over the 5–15 years, for which data were available, the major factors influencing weight gains per ha of sown pastures across all establishment systems were pasture age and stocking rate during the rainy season, including a significant but relatively less important interaction with the establishment system. Performance of animals grazing pastures under moderate management decreased at a modest rate over time. It is inferred that moderate management, and quite possibly, adequate fertilizer application may result in reasonably stable pastures. Further research will verify if these hypotheses are correct.

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Research Paper

Agronomic and molecular characterization of *Chloris gayana* cultivars and salinity response during germination and early vegetative growth

Caracterización agronómica y molecular de cultivares de Chloris gayana *y respuesta a la salinidad en germinación y crecimiento vegetativo temprano*

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Abstract

Chloris gayana is a warm-season grass, often cultivated in areas where soil salinity is a major constraint for forage production. Five cultivars (2 unselected populations and 3 synthetic varieties) were evaluated through agronomic traits as well as Inter Simple Sequence Repeats (ISSRs) and Sequence-related Amplified Polymorphism (SRAP) molecular markers. The consensus between both agronomic and molecular data sets was high (>99%) suggesting that both systems provided similar estimates of genetic relationships. The analysis revealed that synthetic varieties, Finecut, Topcut and Santana, were the most genetically different cultivars, whereas the unselected populations, Pioneer and Katambora, were closely related. Responses to salinity stress during germination and early vegetative growth stages were evaluated in only the synthetic varieties. The results showed that Finecut and Santana were able to germinate in the same proportion as controls even at concentrations of 200 mM NaCl. Under hydroponic conditions, Santana attained approximately 20% higher total dry weight than the other 2 varieties and the longest roots. Finecut presented the highest root dry weight. These results suggested that Santana and Finecut showed high salinity tolerance at germination and early vegetative growth stages, both crucial phases when seeking a successful pasture establishment, particularly in saline environments. Further studies in the field are needed to determine if these hydroponic results are reproduced under field conditions.

Keywords: Molecular markers, morpho-agronomic characters, perennial forage, Procrustes, salinity tolerance.

Resumen

Chloris gayana es una gramínea subtropical, frecuentemente cultivada en áreas donde la salinidad del suelo es una limitación importante para la producción forrajera. En Córdoba, Argentina, fueron evaluadas por sus características agronómicas y moleculares (ISSR y SRAP) cinco cultivares (dos poblaciones no-selectas y tres variedades sintéticas)

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de esta especie. El consenso entre ambos tipos de datos fue alto (>99%) sugiriendo que las caracterizaciones proporcionaron estimaciones similares de las relaciones genéticas. Los resultados mostraron que las variedades sintéticas, Finecut, Topcut y Santana, fueron las más genéticamente diferentes mientras que las poblaciones no-selectas, Pioneer y Katambora, estuvieron estrechamente relacionadas. En las tres variedades sintéticas se evaluó también la respuesta a salinidad en germinación y plántula. Los resultados mostraron que Finecut y Santana germinaron en la misma proporción que los controles, incluso a concentraciones de 200 mM NaCl. En condiciones de hidroponía, Santana alcanzó 20% más de peso seco total y raíces más largas que las otras dos variedades. Finecut presentó el mayor peso seco de raíz. Estos resultados sugieren que Santana y Finecut mostraron alta tolerancia a salinidad en germinación y plántula, etapas cruciales cuando se piensa en el establecimiento exitoso de una pastura, particularmente en ambientes salinos.

Palabras clave: Caracteres morfo-agronómicos, forraje perenne, marcadores moleculares, Procrustes, tolerancia a salinidad.

Introduction

Chloris gayana Kunth, a gramineous perennial species native to Africa, is one of the most important warm-season forage grasses in subtropical and tropical areas of the world (Ponsens et al. 2010). Currently, this species is being introduced into temperate areas as a consequence of increasing minimum temperatures (i.e. less severe winters) because of global warming (Imaz et al. 2015), so its importance as a forage species is increasing world-wide. Its important characteristics are high forage production (up to 15 t DM/ha; see Bogdan 1963), seed production (up to 200 kg/ha; see Bogdan 1963) and some degree of tolerance of major abiotic stresses, such as cold (Cook et al. 2005), drought (Ponsens et al. 2010), flooding (Imaz et al. 2015) and soil salinity (Loch et al. 2004). *Chloris gayana* is a cross-pollinated species that includes diploid as well as tetraploid cytotypes, and the degree of salt tolerance and other agronomic attributes vary among populations or commercial cultivars (Loch et al. 2004).

Different techniques have been used to evaluate genetic variability. Primarily, morphological and agronomic attributes are employed for establishing genetic relationships and to discriminate among populations or commercial cultivars (Loch et al. 2004). More recently, molecular markers have been used for determining genetic variability among and within tetraploid and diploid cultivars/cytotypes (Ubi et al. 2000, 2003). Knowledge of genetic relationships among cultivars is an important consideration for classification and utilization of germplasm resources and breeding (Baretta et al. 2016). When different characterization systems are used, it is necessary to determine the degree of consistency between the different methods. One method used is Generalized Procrustes Analysis (GPA) (Jana et al. 2017). The use of combined datasets has been recommended because each dataset provides complementary information allowing greater power of resolution in genetic variation analysis, and this has proved to be superior to other methods for characterizing plant germplasm (Alves et al. 2013). However, a comparative analysis (consensus) using molecular and agronomic data has not been reported for *Chloris gayana*.

Salinization is one of the oldest and most severe environmental problems in the world, affecting more than 930 M ha in more than 100 countries (Bazihizina et al. 2012). Saline soils can limit establishment, persistence and forage production, especially for perennial pasture species, by affecting plant growth at various stages of development, including germination, seedling emergence and vegetative growth (Munns and Tester 2008). Ability to germinate is particularly important in perennial grasses with small seeds, e.g. *Chloris gayana*, for successful pasture establishment in saline soils (Quiroga et al. 2016). In addition, early vegetative growth in forage grasses is usually constrained under saline conditions (De Luca et al. 2001). Understanding plant responses to high salinity stress and subsequently selecting or developing salt-tolerant varieties can be a solution for increasing livestock production in marginal areas for agriculture, with restrictive soil-climatic conditions (Ashraf and Akram 2009).

Therefore, the objectives of this work were to: 1) characterize diploid unselected populations and synthetic varieties of *Chloris gayana* through agronomic traits and molecular markers; 2) establish the degree of consensus between groupings based on agronomic and molecular data; and 3) evaluate responses to salinity stress at germination and in early vegetative growth stages in the diploid synthetic varieties of *C. gayana*.

Materials and Methods

Agronomic characterization

The experiment was conducted in an experimental field located in Córdoba, Argentina (31º28' S, 64º14' W; 600 masl) on a loamy sand corresponding with an Entic Haplustoll soil, with 2.5% organic matter and pH 7.1. Five diploid *C. gayana* cultivars were tested: 2 unselected populations (Pioneer and Katambora) and 3 synthetic varieties (Topcut, Finecut and Santana). Topcut and Finecut are cultivars selected from Pioneer and Katambora, respectively, for hay production in Australia (Loch et al. 2004), whereas cv. Santana was obtained from *C. gayana* accessions derived from West Africa for salt tolerance in Argentina (Ribotta et al. 2013). All of these materials are used as commercial cultivars in Argentina.

Forty plants of each cultivar were transplanted to a field in a randomized complete-block design with 4 replications of 10 plants each. Distance between plants in a plot and between plots was 1 m. Twenty-five agronomic traits were measured (Table 1).

Molecular characterization

Total genomic DNA was extracted from leaves of each cultivar using the Nucleon PhytoPure Genomic DNA Extraction Kit (GE Healthcare Life Science, Amersham, UK). For Sequence-related Amplified Polymorphism (SRAP) markers, 20 primer pair combinations were tested; they were designed from previous work in other plant species (Li and Quiros 2001; Castonguay et al. 2010). Polymerase chain reactions (PCRs) were performed in volumes of 20 µL, using a protocol modified from Li and Quiros (2001). A total of 18 Inter Simple Sequence Repeats (ISSRs) primers were tested based on common primer selections reported for other Gramineae species (Gutiérrez-Ozuna et al. 2009; Li et al. 2011). The PCRs were performed in volumes of 20 µL, as described by Gutiérrez-Ozuna et al. (2009) with minor modifications. All PCR reactions were performed on an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany). The amplifications were repeated twice and only clear repetitive bands were used in data analysis. Amplification products were loaded on a 2% agarose gel stained with ethidium bromide (10 mg/ml) and run for 3 h at 50 V. DNA fragments were visualized using a BIORAD, Molecular Imager® Gel Doc™ XR System (USA). The SRAP and ISSR bands were scored as absent (0) or present (1) if visible, regardless of their relative intensity.

Table 1. Agronomic traits used for the study in diploid unselected populations and synthetic varieties of *Chloris gayana*.

Trait	Abbreviation	Description
Morphological		
Flag leaf width (mm)	FLW	Measured on main tillers. Leaf width at 2 cm from the ligule, and leaf length from the ligule to the tip of the blade.
Flag leaf length (cm)	FLL	
Tiller diameter (mm)	TID	Measured at the middle of third lowest internode.
Plant height (cm)	PH	Measured from the soil to the base of the inflorescence in tillers at harvest.
Reproductive		
Number of anthecia	NAS	Evaluated in spikelets.
Basal anthecium length (mm)	BAL	
Length of basal anthecium lemma (mm)	LBAL	
Length of basal anthecium palea (mm)	LBAP	
Caryopsis length (mm)	CL	Evaluated in caryopses.
Caryopsis width (mm)	CW	
Weight of 1,000 caryopses (mg)	1000W	
Yield and quality measured on deferred forage		
Plant weight	PW	Plants were harvested by clipping all material above 20 cm and weighed in situ.
Leaf percentage	L%	Calculated as the quotient between blade+sheath and blade+sheath+stems+inflorescences.
Ruminal disappearance of dry matter (%)	RDDM (WP/L/S)	Evaluated in whole plant (WP), leaves (L) [blade+sheath] and stems (S) [stems+inflorescences]. Samples were oven-dried at 60 ˚C and ground. Evaluated in situ after 48 h of incubation in rumen of 3 fistulated cows using the nylon bag technique (Orskov et al. 1980; Vanzant et al. 1998).
Neutral detergent fiber (%)	NDF (WP/L/S)	Evaluated in whole plant (WP), leaves (L) [blade+sheath] and stems (S) [stems+inflorescences] (ANKOM Technology 2014a; 2014b; 2013, respectively).
Acid detergent fiber (%)	ADF (WP/L/S)	
Acid detergent lignin (%)	ADL (WP/L/S)	

Statistical analysis

The agronomic data were subjected to analysis of variance (ANOVA) and the means were compared by the DGC test (Di Rienzo et al. 2002) at P≤0.05 using InfoStat software (Di Rienzo et al. 2016). In addition, cluster analysis was performed through the unweighted pair-group method using an arithmetic average (UPGMA) algorithm based on the standardized Euclidean distance.

For SRAP and ISSR markers, distance matrix was based on Jaccard coefficients [sqrt (1-S)], and a dendrogram was created using UPGMA. A cophenetic coefficient was computed from the clustering matrix to compare the matrix of genetic similarity and the dendrogram. The number of total (TB), monomorphic (MB) and polymorphic (PB) bands and polymorphic information content (PIC) were calculated for each primer or primer combination.

A Generalized Procrustes Analysis (GPA) was performed using the Gower distance (Gower 1975) to measure consensus between the agronomic and molecular information. A principal component (PC) analysis and Minimum Spanning Tree (MST) were performed on each of the distance matrices.

The above-mentioned analyses were performed using InfoStat (Di Rienzo et al. 2016) and InfoGen (Balzarini and Di Rienzo 2011) software.

Evaluation of salinity response at germination and early vegetative stages

The evaluations of salinity response during germination and early vegetative stages were performed in the synthetic varieties of *C. gayana* (Topcut, Finecut and Santana) due to these materials being the most divergent when agronomic and molecular characterization were realized (analysis consensus).

For the evaluation of salinity response at the germination stage, the assay was performed according to López Colomba et al. (2013) with minor modifications. In brief, seeds of these 3 cultivars were surface-sterilized for 20 min in 10% commercial bleach (NaClO 55 g/L), rinsed 3 times in sterile distilled water and transferred to plastic trays lined with filter paper soaked with each salt treatment. The salt treatments used were 0 mM (control), 100, 200 and 300 mM NaCl (approximately 0, 10, 20 and 30 dS/m, respectively). Seeds were incubated in a growth chamber (LIADE, Laboratorio de Investigación Aplicada y Desarrollo, Córdoba, Argentina) under the following conditions: 8 h light/16 h dark photoperiod (55 μmol/m^2/s) at alternating temperatures of 25 ºC (8 h) and 20 ºC (16 h). Four replicates of 30 caryopses per treatment and per cultivar were used. Germinated seeds were counted every 72 h for 21 days, discarded after counting and the proportion of germinated seeds was calculated.

To evaluate salinity response during early vegetative growth, the assay was conducted in a greenhouse under hydroponic conditions. Seedlings with 4 leaves developed were placed individually in holes of a Styrofoam board. These boards were set on rectangular plastic trays (30 × 20 × 60 cm) filled with aerated Hoagland nutrient solution (Hoagland and Arnon 1950). Eighty seedlings of each cultivar were randomly located in 4 trays, with 20 seedlings per tray; each tray was treated as an experimental unit. Two trays were allocated to the control (nutrient solution without NaCl) and the two other trays, to the saline treatment of 400 mM NaCl. Salinization was accomplished by gradually adding 100 mM NaCl every 24 h to avoid osmotic shock, until a concentration of 400 mM NaCl (approximately 40 dS/m) was reached. After 30 days of treatment, the seedlings were evaluated for the following traits: aerial, root and total fresh weight (AFW, RFW and TFW, respectively); aerial, root and total dry weight (ADW, RDW and TDW, respectively); root length (RL); number of stems (SN); and plant height (PH). The seedlings were dried at 60 ºC in a forced air oven for 48 h to achieve stable weight.

Statistical analysis

Effects of cultivar, salt concentration and their interactions on the salt tolerance at germination and early vegetative growth stages were determined by Generalized Linear Mixed Models to estimate differences among cultivars and salt concentrations. The cultivars, salt concentrations and their interactions were considered fixed effects, whereas each repetition was considered a random effect. The average values were compared using Fisher´s LSD test. The above-mentioned analyses were performed using InfoStat (Di Rienzo et al. 2016) software.

Results

Agronomic and molecular characterization

Twenty-two agronomic traits revealed differences among cultivars (Table 2) and the most discriminating traits were those related to morphological and reproductive aspects.

Flag leaf width, flag leaf length and tiller diameter were greatest in Topcut and least in Santana (Table 2). Regarding plant height, the unselected populations (Pioneer and Katambora) were shorter than the synthetic varieties (Topcut, Finecut and Santana; Table 2). In general, Santana stood out above the remainder in terms of reproductive characters. It displayed greater numbers of anthecia, basal anthecium length and length of basal anthecium lemma than most other cultivars, whereas Pioneer showed the lowest values for the BAL, LBAP and LBAL. Caryopsis width, caryopsis length and weight of 1000 caryopses also demonstrated differences between cultivars (Table 2).

In relation to fodder yield, Katambora showed significantly lower plant dry matter (DM) than the remaining 4 cultivars (Table 2). The important indicator of potential feeding value, leaf percentage, was significantly higher in Pioneer and Topcut (mean 63.4%) than in the other 3 cultivars (mean 55.1%). In relation to ruminal disappearance of DM, the disappearance of whole plant material for Finecut (53.5%) was lower than for the remainder (mean 57.6%), while disappearance of leaf was less in Katambora (59.7%) than in the other cultivars (mean 61.4%) (Table 2). Disappearance of stem was lower in Topcut and Finecut (mean 44.8%) than in Santana, Katambora and Pioneer (mean 47.4%). Finecut had the highest percentages of acid and neutral detergent fiber in whole plant and stems. Topcut showed the highest values for acid detergent lignin in stems, while Santana showed the lowest value in leaves (Table 2).

Table 2. Means of 4 replicates (± s.d.) of agronomic traits measured in diploid unselected populations and synthetic varieties of *Chloris gayana*.

Trait	P	Katambora	Pioneer	Finecut	Topcut	Santana
Morphological						
FLW (mm)	***	5.59±0.89 b	5.45±0.85 b	5.61±0.81 b	6.87±1.10 a	5.13±0.71 c
FLL (cm)	***	20.28±4.17 b	18.15±3.36 c	20.39±4.28 b	22.15±3.88 a	18.46±4.45 c
TID (mm)	***	2.64±0.61 b	2.75±0.75 b	2.89±0.70 b	3.29±0.82 a	2.40±0.58 c
PH (cm)	***	101.60±9.94 b	93.06±7.91 c	108.13±11.8 a	107.79±11.7 a	106.42±9.65 a
Reproductive						
NAS	***	3.14±0.50 b	2.89±0.39 c	3.15±0.55 b	3.01±0.41 c	4.08±0.62 a
BAL (mm)	***	3.18±0.31 c	3.05±0.28 d	3.25±0.33 c	3.38±0.33 b	3.53±027 a
LBAP (mm)	***	2.89±0.35 b	2.80±0.28 c	2.98±0.34 b	3.17±0.26 a	3.23±0.26 a
LBAL (mm)	***	3.17±0.36 c	3.01±0.27 d	3.20±0.32 c	3.35±0.35 cb	3.49±0.33) a
CW (mm)	**	0.53±0.08 b	0.54±0.08 a	0.57±0.08 a	0.52±0.07 b	0.55±0.06 a
CL (mm)	***	1.51±0.19 d	1.70±0.24 c	1.82±0.22 b	1.96±0.35 a	1.82±0.21 b
1000W (mg)	***	192.67±9.61 c	255.14±6.44 b	274.00±6.96 a	261.14±5.27 b	278.25±6.65 a
Fodder yield and quality						
L%	**	54.73±9.31 b	62.18±7.64 a	52.79±9.82 b	64.60±7.84 a	57.67±10.47 b
PW (kg per plant)	***	0.30±0.06 b	0.45±0.08 a	0.55±0.15 a	0.48±0.13 a	0.52±0.12 a
RDDM-WP (%)	***	55.26±1.52 a	56.21±3.06 a	53.48±2.15 b	56.01±1.44 a	55.28±1.40 a
NDF-WP (%)	*	73.96±0.97 b	73.56±0.92 b	75.43±0.77 a	73.23±0.70 b	74.63±0.55 a
ADF-WP (%)	*	39.84±1.04 b	38.90±0.96 b	41.70±0.89 a	39.11±1.00 b	40.13±0.64 b
ADL-WP (%)	n.s.	4.13±0.36	4.50±0.02	4.55±0.22	4.37±0.10	4.44±0.16
RDDM-L (%)	*	59.67±2.71 b	61.17±1.63 a	61.21±1.60 a	61.93±1.58 a	61.00±1.47 a
NDF-L (%)	n.s.	70.25±1.39	71.13±1.13	72.15±1.25	71.20±0.84	69.93±0.32
ADF-L (%)	n.s.	35.64±0.32	35.92±0.68	36.46±0.95	35.79±0.74	35.09±0.36
ADL-L (%)	**	3.00±0.08 a	2.71±0.20 a	2.96±0.31 a	2.77±0.09 a	2.42±0.13 b
RDDM-S (%)	***	47.00±2.26 a	47.62±1.52 a	45.06±2.46 b	44.62±1.71 b	47.45±1.78 a
NDF-S (%)	**	79.28±0.59 b	80.40±0.88 a	81.18±0.96 a	79.42±0.28 b	79.57±0.56 b
ADF-S (%)	*	45.44±0.42 b	45.74±0.83 b	47.48±1.26 a	46.04±0.96 b	44.71±0.57 b
ADL-S (%)	**	6.02±0.57 b	6.17±0.14 b	6.54±0.80 b	7.11±0.38 a	5.83±0.11 b

Abbreviations: FLW (Flag leaf width), FLL (Flag leaf length), TID (Tiller diameter), PH (Plant height), NAS (Number of anthecia), BAL (Basal anthecium length), LBAP (Length of basal anthecium palea), LBAL (Length of basal anthecium lemma), CW (Caryopsis width), CL (Caryopsis length), 1000W (Weight of 1,000 caryopses), L% (leaf percentage), PW (Plant weight), RDDM (Ruminal disappearance of dry matter), NDF (Neutral detergent fiber), ADF (Acid detergent fiber), ADL (Acid detergent lignin). For RDDM, NFD, ADF and ADL, companion letters mean WP (whole plant), L (leaves: leaf blades plus sheaths) and S (stems plus inflorescences).

As for molecular characterization, 20 SRAP primer combinations showing reproducible and polymorphic patterns generated a total of 256 scorable bands with an average of 12.8 bands per primer combination, of which 82 were polymorphic (32.0%). The total number of bands produced by each primer combination ranged from 4 (F9/R8) to 22 (F13/R15), while the number of polymorphic bands produced by each primer combination ranged from 1 (ME2/R9) to 8 (F13/R15 and ME4/R8) (Table 3); ME2/EM2 and ME2/R9 were the primer combinations with more polymorphic information content (PIC) (0.36). It is noteworthy that Finecut, Santana and Topcut showed numerous unique markers (17, 8 and 3, respectively), whereas Pioneer and Katambora showed none. Eighteen ISSR primers amplified a total of 306 bands, of which 147 were polymorphic (48.0%) with an average of 17 bands per primer. The number of polymorphic bands ranged from 4 (UBC864, UBC862 and UBC826) to 15 (SC ISSR1); UBC864 and D12 ISSR had the highest PIC (0.36) (Table 3). Cultivars Finecut, Topcut and Santana showed numerous unique markers (25, 14 and 8, respectively), which was similar to the pattern observed for SRAP markers.

A dendrogram based on UPGMA analyses of the 25 agronomic traits revealed that the 5 cultivars constituted 2 major clusters (Figure 1a; genetic similarity coefficient range 4.65–7.70; cophenetic correlation coefficient = 0.97). Cultivar Finecut, which formed a separate cluster, was genetically different from the remaining cultivars, which formed a second group. While Pioneer and Katambora were closely related, Santana was intermediate between them and Topcut (Figure 1a). The dendrogram based on UPGMA analyses of the SRAP (genotypic) data showed a similar trend to that obtained with agronomic analysis (Figure 1b; genetic diversity coefficient range 0.24–0.46; cophenetic correlation coefficient = 0.95). Finally, the ISSR dendrogram exhibited a trend to form clusters similar to those based on agronomic traits and SRAP markers, with the exception of Topcut, which showed close relationship with Pioneer and Katambora (Figure 1c; genetic diversity coefficient range 0.33–0.59; cophenetic correlation coefficient = 0.99).

Generalized Procrustes Analysis showed that: 1) 71.5% of the total variability contained in the datasets was explained by the first 2 axes (PC1: 41.1% and PC2: 30.4%); and 2) there was high correspondence between agronomic and molecular datasets (99.9% consensus) (Figure 2). Cultivars Finecut and Topcut were distinct and different from other cultivars whereas the unselected populations, Pioneer and Katambora, appeared to be closely related. Cultivar Santana showed some relationship with Pioneer and Katambora (Figure 2).

Table 3. Number of polymorphic bands (PB), monomorphic bands (MB), total bands (TB) and polymorphic information content (PIC) revealed by 20 SRAP primer combinations and 18 ISSR primers in diploid unselected populations and synthetic varieties of *Chloris gayana.*

Primer combinations SRAP marker	PB	MB	TB	PIC	ISSR marker	PB	MB	TB	PIC
F9/R8	0	4	4	NA	17899B	8	4	12	0.29
F13/R9	3	5	8	0.27	D12	5	7	12	0.36
F13/R14	5	14	19	0.31	HB13	11	13	24	0.33
F13/R15	8	14	22	0.32	HB15	13	19	32	0.31
F11/EM2	2	3	5	0.27	RAF16	9	10	19	0.3
ME2/R8	6	8	14	0.32	RAF4	9	4	13	0.3
ME2/R9	1	9	10	0.36	SC ISSR1	15	14	29	0.33
ME2/R15	7	11	18	0.31	SC ISSR9	7	9	16	0.3
ME2/R14	3	3	6	0.33	UBC825	12	12	24	0.3
ME2/R7	5	6	11	0.29	UBC834	11	6	17	0.29
ME2/EM2	4	10	14	0.36	UBC808	9	9	18	0.29
ME4/R15	2	6	8	0.32	UBC810	5	12	17	0.31
F9/R14	6	15	21	0.27	UBC816	6	5	11	0.28
ME4/R8	8	11	19	0.33	UBC826	4	7	11	0.32
ME4/EM2	0	10	10	NA	UBC827	8	6	14	0.32
ME4/R7	6	13	19	0.28	UBC861	7	9	16	0.3
ME4/R9	4	8	12	0.29	UBC862	4	6	10	0.34
F9/R7	0	5	5	NA	UBC864	4	7	11	0.36
F10/R9	5	8	13	0.27					
F10/R8	7	11	18	0.31					
Total	82	174	256		Total	147	159	306	

NA: Not available.

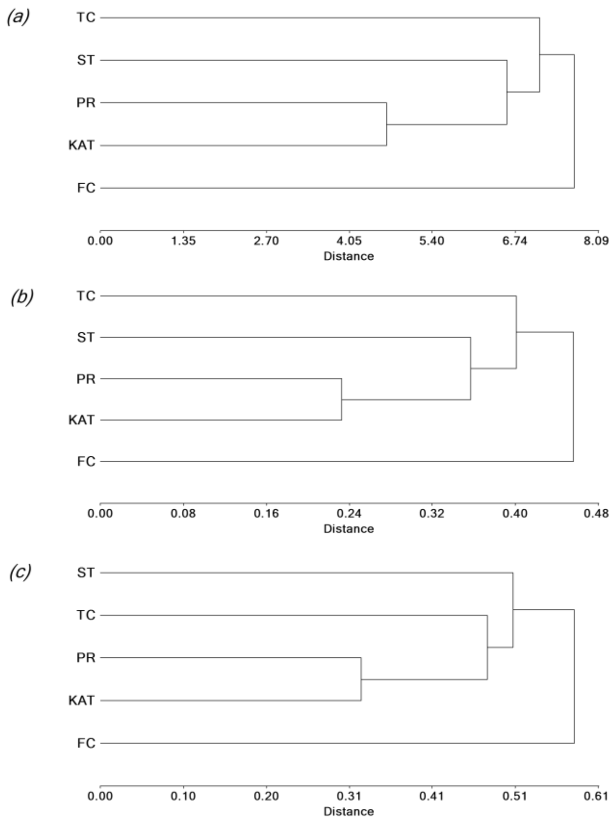

Figure 1. UPGMA dendrogram showing genetic relationships among diploid unselected populations and synthetic varieties of *Chloris gayana*. The relationships were calculated on the basis of genetic distances using: a) 25 agronomic traits; b) 20 SRAP markers; and c) 18 ISSR markers. Katambora (KAT), Pioneer (PR), Finecut (FC), Topcut (TC) and Santana (ST).

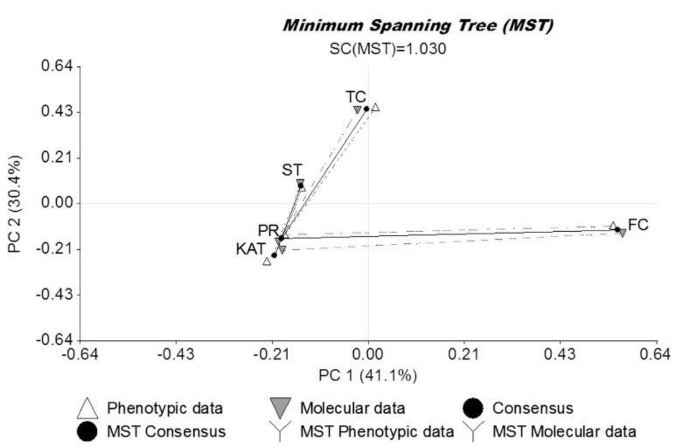

Figure 2. Configuration of consensus matrix from Generalized Procrustes Analysis of diploid unselected populations and synthetic varieties of *Chloris gayana* based on 20 SRAP markers, 18 ISSR markers and 25 agronomic traits. Katambora (KAT), Pioneer (PR), Finecut (FC), Topcut (TC) and Santana (ST).

Evaluation of salinity responses during germination and early vegetative stages

The cultivars responded differentially to salinity in terms of germination percentage with significant cultivar × salt concentration interactions (P = 0.035; Table 4). All cultivars recorded excellent germination rates (mean 96%) in water, and cultivars Finecut and Santana still displayed high values in saline conditions (means of 96 and 93% for 100 mM and 200 mM NaCl solutions, respectively; Table 4). By contrast, germination of cv. Topcut declined to 82% in both 100 and 200 mM NaCl solutions. At the highest saline concentration (300 mM NaCl), all cultivars exhibited reductions in seed germination relative to control (87% for Finecut, 76% for Santana and 72% for Topcut; Table 4).

Table 4. Proportion of germinated seeds of diploid synthetic varieties of *Chloris gayana* at increasing salinity levels.

Treatment	Cultivar		
	Finecut	Topcut	Santana
0 mM NaCl	0.94(±0.06) a	0.94(±0.04) a	0.99(±0.02) a
100 mM NaCl	0.93(±0.05) a	0.82(±0.08) b	0.99(±0.01) a
200 mM NaCl	0.93(±0.07) a	0.82(±0.09) b	0.92(±0.04) a
300 mM NaCl	0.87(±0.03) b	0.72(±0.15) b	0.76(±0.14) b

Mean values of 4 replicates (± s.d.) are presented. Different letters indicate significant differences (P<0.05) by LSD Fisher´s test.

In terms of early vegetative growth, cultivars responded differentially to 400 mM NaCl in aerial dry weight (ADW), root dry weight (RDW), total dry weight (TDW), maximum root length (MRL) and plant height (PH) (P<0.05; Table 5). For all parameters other than ADW, the interaction cultivar × treatment was significant (P<0.05). Under stressful saline conditions, Finecut showed less depression in growth than Santana and Topcut. ADW of Finecut in saline solution was 55.1% of that in straight water, while corresponding values for Santana and Topcut were 51.4 and 44.8%, respectively. Similarly, RDW for Finecut in saline solution was 80% of that in water, while values for Santana and Topcut were 56 and 52%. This resulted in TDW values in saline solution relative to straight water for Finecut, Santana and Topcut of 58.8, 50.4 and 46.9%, respectively. Despite these outcomes, in absolute terms Santana produced more TDW in saline solution than Finecut and Topcut. Topcut was the most sensitive in reaction to salinity, showing the largest reductions in aerial dry weight, root dry weight, total dry weight, plant height and maximum root length (Table 5).

Table 5. Plant growth parameters for 30 days after germination of diploid synthetic varieties of *Chloris gayana* under non-saline (0 mM NaCl) and saline (400 mM NaCl) conditions in hydroponic culture. Means of 2 replicates (40 plants) (± S.D.) for aerial dry weight (ADW), root dry weight (RDW), total dry weight (TDW), number of stems (SN), plant height (PH) and maximum root length (MRL).

Variable	Non-saline (0 mM NaCl)			Saline (400 mM NaCl)			Cultivar × treatment interaction
	Finecut	Topcut	Santana	Finecut	Topcut	Santana	
ADW (g)	1.74±0.66B	2.03±0.67AB	2.14±0.71A	0.96±0.27AB	0.91±0.29B	1.10±0.29A	P=0.1196
RDW (g)	0.20±0.06b	0.25±0.10a	0.25±0.09a	0.16±0.06c	0.13±0.04d	0.14±0.05d	P=0.0008
TDW (g)	1.87±0.61b	2.28±0.72a	2.56±0.77a	1.10±0.35d	1.07±0.39d	1.29±0.44c	P=0.0166
SN	3.17±1.43b	3.94±1.31a	4.54±1.57a	3.41±1.07b	2.94±1.34b	3.39±1.58b	P=0.0034
PH (cm)	132.94±16.4a	125.27±16.2b	114.33±14.3c	78.12±10.6d	70.58±14.4e	74.02±12.2e	P=0.0003
MRL (cm)	34.54±5.92c	46.39±9.41a	41.33±6.52b	35.68±6.84c	36.78±5.80c	40.51±6.68b	P<0.0001

Lower-case letters show differences among cultivar × treatment combinations for variables where interaction was significant (P<0.05). Uppercase letters show significant differences among cultivars within treatments for variables where interaction was not significant (P>0.05).

Discussion

This study presents 3 major contributions: 1) the characterization of diploid unselected populations and synthetic varieties of *C. gayana* via agronomic traits and SRAP and ISSR molecular markers; 2) the identification of high consensus (>99%) between molecular markers and agronomic traits, suggesting that both systems provided similar estimates of genetic relationships; and 3) novel evidence showing existence of variability in salinity tolerance among diploid synthetic varieties of *C. gayana*.

Agronomic characterization allowed us to differentiate between the evaluated cultivars. While Agnusdei et al. (2009) suggest there is a negative association between leaf dimensions and nutritive quality, cv. Topcut showed the highest values for traits related to leaf dimensions in our study without detriment to its nutritional value. In a study conducted in *Cenchrus* spp. by Griffa et al. (2012), the morphological traits width and length of flag leaf and 1,000-seed weight were closely related to seed production. In fact, seed production was negatively correlated with leaf size but positively correlated with 1,000-seed weight. Our data support this relationship as cv. Santana exhibits low values for flag leaf length and width, but high values for seed-related characters. This relationship was confirmed by Cicetti et al. (2015) in a comparative study with other *C. gayana* diploid cultivars. The good performance of seed-related traits (weight, width and length) observed in Santana and Finecut suggests their potential for producing vigorous seedlings and enhanced performance at early stages of pasture establishment (Giordano et al. 2013). The results for fiber percentage and ruminal disappearance of dry matter were within the range of values reported for deferred *C. gayana* (Otondo 2004; Borrajo et al. 2015). These observations have significance for deferred forage utilization (Gargano et al. 2001).

In recent years, ISSR and SRAP markers have been recognized as low-cost useful molecular techniques in marker-assisted selection, for genetic linkage map construction and genetic diversity analysis (Castonguay et al. 2010; Shao et al. 2010). Our results suggest that ISSRs were superior to the SRAP markers in their capacity to produce polymorphic bands, which is in agreement with previous reports for *Chrysanthemum morifolium* (Shao et al. 2010), *Cynodon arcuatus* (Huang et al. 2013) and *Prunus armeniaca* (Li et al. 2014). The differences between the methods might be related to the fact that, unlike ISSRs, which are targeted to micro-satellite regions in the whole genome, SRAP markers preferentially amplify open reading frames targeting functional regions of the genome (see Li and Quiros 2001).

It was interesting that clustering obtained from molecular and agronomic data revealed a grouping where the most closely related were the unselected populations, while the most genetically different cultivars were those obtained from breeding programs (Finecut, Topcut and Santana). The relationships between cultivars Pioneer and Katambora revealed by the analysis of ISSR and SRAP techniques are consistent with results reported by Pérez et al. (1999) using RAPD markers.

The information obtained by Generalized Procrustes Analysis (GPA) allowed us to establish a high consensus between the different data sets (agronomic and molecular) and suggested that both systems provided similar estimates of genetic relationships. Similar results were described by Bermejo et al. (2010) in the evaluation of recombinant inbred lines of lentils. In our study, the high degree of consensus obtained is probably a function of the high number of traits and molecular markers used. Even though GPA proved to be a very efficient tool to show the relationships among relative ordinations of a single genotype under different types of markers, it does not mean that it can replace/substitute for the information provided by each. The knowledge of quantitative and molecular variability can optimize the work of plant breeders (Ribotta 2011).

Seed germination is a critical phase when considering pasture establishment. Salinity imposes stresses on seeds during the germination process as soil water availability is reduced due to the low osmotic potential. Therefore, imbibition and germination of seeds can be constrained at increasing soil salinity (Munns and Tester 2008; Quiroga et al. 2016). Significantly, in this study differences were found among synthetic varieties under saline conditions. Finecut and Santana were able to germinate in the same proportion as controls even at concentrations of 200 mM NaCl, while germination in Topcut was suppressed at 100 mM NaCl. Curiously, Finecut and Santana have heavier seeds than Topcut, which might provide higher amounts of reserves to sustain germination under stressful conditions (Hanley et al. 2007). Grieve and Francois (1992) indicated that larger seeds frequently confer distinct advantages, e.g. seedling vigor and hardiness, to a species in overcoming salinity.

Germination and seedling establishment are considered the most critical stages of the plant life cycle under saline conditions. Salt tolerance in these ontogenic stages would confer distinct advantages to a species in terms of persistence and good performance in saline environments (Bazzigalupi et al. 2008). In a study conducted in *Chloris gayana*, salt tolerance conferred higher DM production (Moore et al. 2004), and provided higher numbers of tillers (De Luca et al. 2001). In our work, Santana showed better performance in total dry weight than Topcut or Finecut, whereas Finecut was the only cultivar that did not reduce its number of stems in saline conditions. These characteristics would allow survival of Santana and Finecut cultivars under saline conditions in the field. The root system plays a fundamental role in plant growth and survival because of water and nutrient uptake (Wang et al. 2009). It is known that plants will allocate relatively more biomass to roots if the limiting factor for growth is below ground (e.g. nutrients, water) (Poorter et al. 2012). In this work, Santana showed the longest roots, which would facilitate exploitation of mobile nutrients. According to Zolla et al. (2010), this characteristic should allow plants to compete better for resources under suboptimal soil conditions. Further analyses may be necessary to give insights to the mechanisms involved in the better response of Santana to salt stress under hydroponic conditions.

Summarizing, this study provides valuable information about agronomic and molecular characterization, which enables us to detect the existence of differences between unselected populations and synthetic varieties of *C. gayana*. Moreover, the agronomic and molecular characterization showed high consensus, suggesting similar estimates of the variability among cultivars. Our assessment of the 3 synthetic varieties in terms of their likely usefulness in saline environments suggests that Finecut and Santana show most promise for inclusion in forage planning exercises, given their high salinity tolerance at germination and early vegetative growth stages, both crucial phases when considering successful pasture establishment. Further field studies will be necessary to verify the hydroponic glasshouse results obtained in this study.

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Research Paper

RETRACTED: Agro-morphological characterization of *Urochloa* grass accessions in Kenya

Caracterización agro-morfológica de accesiones de Urochloa *en Kenia*

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RETRACTED

Abstract

Information of existing phenotypic diversity of *Urochloa* grass is important in selection for pasture development. Forty-seven accessions from 8 different *Urochloa* species obtained from the genebank of International Center for Tropical Agriculture in Colombia were characterized using a set of 22 agronomic and morphological characters. Most of the accessions originated from the East African region. The accessions were planted in December 2013 at Katumani, Eastern Kenya. Twelve seedlings of each accession were transplanted in single row plots at a spacing of 10 cm between plants. Agro-morphological data were collected from the middle 10 plants for each accession. Multivariate analyses were applied to cluster the accessions with similar agronomic and morphological traits. Principal component analysis revealed 4 components with eigenvalues greater than 1 with first and second components accounting for 23.8 and 20.2% of variation, respectively. The cluster analysis identified 5 main groups differentiated largely by days to 50% flowering, flowering duration and plant spread. Leafiness, growth habit, culm thickness and stigma color did not show significant difference among the clusters. The results provided useful information on the diversity in agronomy and morphology that exists among accessions but the collection was not sufficiently diverse and a much wider sample of accessions is needed to identify the true extent of variation in this genus. Important variables like dry matter yield and chemical composition of these accessions would need to be assessed before proceeding with any further evaluation in the field.

Keywords: *Brachiaria*, cluster analysis, germplasm, principal component analysis, tropical grass.

Resumen

En Katumani, Kenia Oriental, se caracterizaron 47 accesiones de ocho especies diferentes del género *Brachiaria* (ahora: *Urochloa*) obtenidas del banco de germoplasma del Centro Internacional de Agricultura Tropical (CIAT) en Colombia, utilizando un conjunto de 22 caracteres agronómicos y morfológicos. La mayoría de las accesiones se originaron en África Oriental. Para el efecto fueron trasplantadas 12 plántulas en parcelas de una línea a una distancia de 10 cm entre ellas. Las observaciones se realizaron en 10 plantas y para el análisis de los datos se utilizaron métodos multivariados con el fin de agrupar las accesiones con características agronómicas y morfológicas similares. El análisis de componentes principales (ACP) permitió identificar cuatro componentes con eigenvalores >1, donde el primero y segundo representaron 23.8 y 20.2% de variación, respectivamente. En el análisis de conglomerados fueron identificados cinco grupos principales, diferenciados por las características: días a 50% de floración, duración de floración y despliegue de la planta. La frondosidad, el hábito de crecimiento, el grosor del culmo y el color del estigma no mostraron diferencias significativas entre los grupos. Los resultados proporcionaron información útil sobre la diversidad agronómica y morfológica entre las accesiones, pero el tamaño reducido de la colección no permitió identificar el alcance de la

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variación en este género. Variables importantes como el rendimiento de materia seca y la composición química de estas accesiones deben ser evaluadas antes de proceder con trabajos similares en campo.

Palabras clave: Análisis de componentes principales, análisis de conglomerados, *Brachiaria*, germoplasma, gramíneas tropicales.

Introduction

The genus *Brachiaria* consists of more than 100 species with 33 species having been reported in Kenya. Eastern and Central Africa is the center of origin and diversity of the *Brachiaria* grasses (Boonman 1993). However, only a few of the species have been selected for forage production and are widely cultivated in South America, Australia and Southeast Asia. Taxonomically, they are now accepted to belong to the genus *Urochloa* by the major taxonomic databases such as GRIN (2018). The most common and extensively cultivated species for pastures are *U. brizantha* (Hochst. ex A. Rich.) R.D. Webster [syn. *B. brizantha* (Hochst. ex A. Rich.) Stapf], *U. ruziziensis* (R. Germ. & C.M. Evrard) Crins (syn. *B. ruziziensis* R. Germ. & C.M. Evrard), *U. decumbens* (Stapf) R.D. Webster (syn. *B. decumbens* Stapf) and *U. mutica* (Forssk.) T.Q. Nguyen [syn. *B. mutica* (Forssk.) Stapf] (Ndikumana and de Leeuw 1996). From early 1940s to mid1980s, there were several major and minor collection missions by several research institutions targeting *Urochloa* species in Eastern Africa (Keller-Grein et al. 1996). Most of this germplasm is held in genebanks under the International Treaty on Plant Genetic Resources for Food and Agriculture and is used in evaluation and breeding of cultivars.

Despite the diversity of *Urochloa* spp. in Eastern and Central Africa, comparatively little information is available on their agro-morphological characteristics. In Kenya, past evaluations identified and selected Congo Signal grass (*U. ruziziensis*) for commercialization in the Western region (Wandera 1997). However, demand for seed was comparatively low compared with other preferred grasses, e.g. Rhodes grass *(Chloris gayana* Kunth); consequently, seed production was discontinued. Other species, e.g. *U. brizantha*, *U. decumbens* and *U. humidicola* (Rendle) Morrone & Zuloaga [syn. *B. humidicola* (Rendle) Schweick.], were evaluated in small-plot agronomic trials in the 1990s (Ndikumana and de Leeuw 1996) but none has been used commercially in Africa.

In recent years, there has been renewed interest in Kenya to develop high-yielding and nutritious forages to support the growing livestock industry using *Urochloa* species. Grasses in the genus *Urochloa* have advantages over grasses in other genera including adaptation to infertile acid soils and high dry matter yields (Rodrigues et al. 2014). The recent program on pasture development in Kenya commenced with introduction of selected lines, improved cultivars and hybrids from South America and Australia to assess their adaptation and production in different agro-ecological zones. Unfortunately, some of these grasses have shown susceptibility to pests and diseases (Njarui et al. 2016). Consequently, there is a need to explore other germplasm either through collection or acquisition of material maintained by different research institutions and genebanks across the world.

The Kenya Agricultural and Livestock Research Organization (KALRO) obtained a selection of *Urochloa* accessions from the Genetic Resources Program of International Center for Tropical Agriculture (CIAT). To exploit this germplasm for forage, it is important to understand the agro-morphological characteristics and variations that exist among the accessions. Past evaluations of collections of various tropical genera and species have indicated considerable diversity in growth habit (Veasey et al. 2001; van de Wouw et al. 2009). Morphological and agronomic classification methods have been widely used to group accessions with similar characters (Pengelly et al. 1992; van de Wouw et al. 1999a). Successful classification of large numbers of accessions of buffel grass (*Cenchrus ciliaris* L.), guinea grass (*Panicum maximum* Jacq.) and *Indigofera* spp. (Hassen et al. 2006; Jorge et al. 2008; van de Wouw et al. 2008) using cluster and principal component analyses has identified distinct groups. The objective of this study was to characterize the *Urochloa* grass accessions obtained from CIAT and determine the level of diversity in morphological and agronomic traits, which can be exploited for possible integration in different farming systems of Kenya.

Materials and Methods

Site

The experiment was conducted from December 2013 to August 2014 at KALRO - Katumani (37º28' E, 1º58' S;

1,600 masl), Kenya. The climate and soil characteristics have been described by Njarui and Wandera (2004). Mean annual rainfall is 717 mm, with a bimodal pattern; the long rains occur from March to May and the short rains from mid-October to December with peaks in April and November, respectively. There are 2 distinct dry seasons, a short dry spell in January-February and a long dry season from June to mid-October. Evapotranspiration rates are high and exceed the amount of rainfall in all months except November, when total rainfall exceeds evaporation. The mean monthly temperature is 19.6 ºC with March (21 ºC) and July (16.6 ºC) being the warmest and coolest months, respectively. Soils are chromic luvisols (Aore and Gitahi 1991) and are generally low in nitrogen and phosphorus (Okalebo et al. 1992), with a pH of 6.5.

Treatment and design

Individual seeds of 80 *Urochloa* accessions, including one commercial cultivar, were sown in polybags in a greenhouse using a mix of forest soil, sand and manure at a ratio of 3:2:1. At about 4 weeks after seedling emergence, in December 2013, 12 uniform and healthy seedlings from each accession were transplanted in the field. Accessions were randomly allocated in unreplicated single rows without following the order of accession numbers. The spacing between seedlings within rows was 10 cm and the inter-row spacing was 2 m, while the space between different accessions within rows was 1 m. Triple superphosphate (TSP with 46% P_2O_5) fertilizer was applied at a rate of 40 kg P/ha only during planting. The plots were kept weed-free by hand-weeding.

Origin of materials

As no *Urochloa* grass seed collections were available in the Kenyan genebank, KALRO obtained the accessions screened in the experiment from the Genetic Resources Program of CIAT, Colombia. Eighty accessions of *Urochloa* spp. were supplied under the Standard Material Transfer Agreement of FAO. The majority of the accessions supplied had been collected in the Eastern region of Africa, mainly in Ethiopia and Kenya.

Data collection

Data were collected from only 47 accessions comprising 8 species listed in Table 1. The remaining 32 accessions and *U. brizantha* cv. Toledo (CIAT No. 26110) are not listed since they failed to maintain the minimum number of plants required for monitoring due to poor establishment and termite damage, occasioned by an unexpected short dry spell. Twenty-two agro-morphological characters were measured (Table 2), based on their agronomic relevance and expected variation among accessions. These qualitative characters were recorded for 10 plants for each accession as suggested by van de Wouw et al. (1999b) discarding one plant on each end of the row to avoid any border effects. All plants were evaluated once at 50% flowering stage to minimize differences due to stage of growth. The data were collected for a single season in order to minimize differences due to environment of the characterization site as recommended by van de Wouw et al. (1999b).

Data analyses

The correlations among the observed variables were calculated using the Pearson's correlation coefficient. When pairs of variables had a high correlation coefficient ($r \geq 0.7$), one of these variables was omitted to avoid indirect weighting in cluster analysis according to criteria applied by Hassen et al. (2006) and van de Wouw et al. (2009). After standardizing the variables to a mean of 0 and a variance of 1, a principal component analysis was carried out using the program Statistical Analysis System (SAS) software (SAS 2001). Hierarchical cluster analysis was carried out using the complete linkage method according to criteria recommended by van de Wouw et al. (2009). Variations between the groups of accessions for the different characteristics were assessed by one-way analysis of variance considering groups as treatments and individual accessions within a group as replications.

Table 1. *Urochloa* accessions used in the characterization study.

Accession No.[1]	Species	Origin	Location	Latitude (º)	Longitude (º)	Elevation (masl)
26107	*Urochloa. brizantha*	Burundi	Rutana	4.0167 S	30.0833 E	1,220
26129	*U. brizantha*	Burundi	Rutana	3.9667 S	30.15 E	1,170
26133	*U. brizantha*	Burundi	Rutana	4.0167 S	30.0833 E	1,200
26647	*U. brizantha*	Burundi	Karuzi	3.05 S	30.15 E	1,640
16106	*U. brizantha*	Ethiopia	Shoa	8.9833 N	37.3333 E	1,900
16118	*U. brizantha*	Ethiopia	Welega	9.0833 N	35.9 E	1,890
16122	*U. brizantha*	Ethiopia	Welega	9.55 N	35.45 E	1,990
16150	*U. brizantha*	Ethiopia	Sidamo	7.15 N	37.95 E	2,040
16158	*U. brizantha*	Ethiopia	Sidamo	6.8167 N	37.7167 E	1,990
16169	*U. brizantha*	Ethiopia	Harerge	9.4 N	42.0333 E	1,970
16289	*U. brizantha*	Ethiopia	Kaffa	8.1 N	37.4667 E	1,850
16320	*U. brizantha*	Ethiopia	Welega	8.9333 N	35.5333 E	1,640
16324	*U. brizantha*	Ethiopia	Gojjam	10.9667 N	36.4833 E	1,690
16339	*U. brizantha*	Ethiopia	Gonder	12.5167 N	37.0339 E	2,080
36083	*U. humidicola*	Ethiopia	Sidamo	5.86 N	39.1 E	1,790
6130	*U. ruziziensis*	Kenya	Rift Valley	0.6167 N	35.1667 E	2,030
6384	*U. brizantha*	Kenya	Rift Valley	0.0667 S	34.6833 E	1,400
6385	*U. brizantha*	Kenya	Rift Valley	0.6 N	35.5333 E	2,120
6399	*U. brizantha*	Kenya	Rift Valley	-	-	2,130
6426	*U. brizantha*	Kenya	Rift Valley	0.5833 N	35.3667 E	2,300
6684	*U. brizantha*	Kenya	Rift Valley	0.35 N	34.8167 E	1,606
16482	*U. brizantha*	Kenya	Uashin Gishu	0.5333 N	35.0333 E	1,700
16483	*U. brizantha*	Kenya	Nandi	0.35 N	35.05 E	1,900
16514	*Brachiaria jubata*[2]	Kenya	Trans Nzoia	1.1167 N	35.0667 E	1,920
16536	*B. jubata*	Kenya	Trans Nzoia	1.0667 N	34.8833 E	1,800
16539	*B. jubata*	Kenya	Trans Nzoia	0.8833 N	35.9333 E	1,640
16541	*B. jubata*	Kenya	Nandi	0.35 N	35.05 E	1,900
26302	*U. decumbens*	Rwanda	Byumba	1.3333 S	30.3 E	1,410
26353	*B. jubata*	Rwanda	Byumba	1.4667 S	30.2833 E	1,470
6674	*U. brizantha*	Tanzania	Tanga	5.35 S	37.45 E	-
6241	*U. ruziziensis*	Uganda	-	-	-	909
6686	*U. brizantha*	Uganda	East Mengo	1.4333 N	32.0167 E	1,061
6735	*U. brizantha*	Malawi	Central	13.6833 S	33.75 E	1,300
6419	*U. ruziziensis*	Zaire	-	-	-	-
16097	*U. brizantha*	Zimbabwe	-	-	-	-
16903	*U. nigropedata*	Zimbabwe	Murewa	17.7 S	31.8 E	1,360
16906	*U. nigropedata*	Zimbabwe	Mazowe	17.6333 S	30.95 E	1,240
26894	*U. subquadripara*	Togo	Maritime	6.1667 N	1.25 E	10
26886	*U. lata*	Oman	-	17.1667 N	54.5 E	200
660	*U brizantha*	Unknown	-	-	-	-
664	*U. decumbens*	Unknown	-	-	-	-
667	*U. brizantha*	Unknown	-	-	-	-
6369	*U. humidicola*	Unknown	-	-	-	-
6370	*U. decumbens*	Unknown	-	-	-	-
6711	*U. ruziziensis*	Unknown	-	-	-	-
26646	*U. brizantha*	Unknown	-	-	-	-
26991	*U. brizantha*	Unknown	-	-	-	-

[1]CIAT accession numbers.
[2]Species not listed in GRIN (2018); in TPL (2013) recognized as *Brachiaria jubata* (Fig. & De Not.) Stapf.

Table 2. Characters used in the agronomic and morphological study.

Character	Definition	No. of observations	Unit
Date of first flowering[1]	Appearance of first flower	full plot score	day
Date to 50% flowering	Half of plants in plots have flowered	full plot score	day
Date to full flowering[1]	All plants have flowered	full plot score	day
Flowering duration	Days from first flower to full flowering	full plot score	day
Plant height	Average height from ground to flag leaf at 50% flowering	10 plants	cm
Leafiness	An estimate of the amount of leaves (1 = no leaves to 10 = very leafy at full flowering)	full plot score	1–10
Growth habit	Angle of the culm to the ground (1 = prostrate to 5 = erect), taken at 50% flowering	full plot score	1–5
Culm thickness	Average diameter of culm at lowest internode at 50% flowering	10 observations	mm
Rhizomes[1]	Presence of rhizomes (1 = no rhizomes to 10 prolific rhizomes), 2 weeks after harvest	full plot score	1–10
Leaf length	Length from ligule to tip of leaf (second leaf from flag leaf)	10 observations	cm
Leaf width	Width of leaf at widest point (second leaf from flag leaf)	10 observations	mm
Leaf ratio	Leaf length divided by leaf width		
Ligule length[1]	Length of the ligule	10 observations	mm
Leaf hairiness-adaxial[1]	Hairiness of adaxial surface of the leaf (1 = glabrous to 5 = medium dense hairs)	10 observations	1–5
Leaf hairiness-abaxial[1]	Hairiness of abaxial surface of the leaf (1 = glabrous to 5 = medium dense hairs)	10 observations	1–5
Leaf sheath hairiness	Hairiness of the leaf sheath (1 = glabrous to 5 = medium dense hairs)	10 observations	1–5
Inflorescence length[1]	Length of the main rachis from the lowest branch to the top spikelet/bristles	10 observations	cm
Inflorescence width	Width at widest point	10 observations	cm
Inflorescence ratio[1]	Inflorescence length divided by width		
Raceme length[1]	Longest primary branch of inflorescence	10 observations	cm
Stigma color	1 = no purple to 5 = entire stigma purple	full plot score	1–5
Plant spread	Diameter from one edge to the other of the plant	10 observations	cm

[1]Characters excluded from agro-morphological analysis due to high correlation (Pearson's coefficient ≥0.7) with other characters.

Results

The principal component (PC) analysis revealed 4 components with eigenvalues greater than 1 (Table 3). The first PC, which explained 23.8% of the total variation, was strongly and positively associated with agro-morphological characters: leaf width (r = 0.76, P<0.0001), plant height (r = 0.75, P<0.0001), days to 50% flowering (r = 0.74, P<0.0001) and plant spread (r = 0.74, P<0.0001). The second PC, which explained 20.2% of the total variation, was strongly and positively associated with leaf length (r = 0.88, P<0.0001), inflorescence width (r = 0.76, P<0.0001) and leaf ratio (r = 0.68, P<0.0001). The third PC, which explained 14.5% of the total variation, was positively associated with days to 50% flowering (r = 0.56, P<0.0001) and flowering duration (r = 0.68, P<0.0001), while the fourth PC, which explained only 9.9% of the total variation, was strongly and positively associated with culm thickness (r = 0.71, P<0.0001). The first and second PCs are plotted in Figure 1 and described 44% of the variation. They revealed separation of groups across the PC1 axis. Accessions with higher values for PC1 (CIAT 16320, 6674, 6686, 6369, 6130, 26133, 6419, 667 and 660) had a prostrate growth habit, greater plant spread and were taller and late flowering. Accessions with higher values for PC2 had an erect growth habit, large leaf ratio and higher inflorescence width.

Most of the plant characters recorded showed significant (P<0.05) variations between accessions (Table 4). The differences in days to 50% flowering were large and varied from 91 to 194 days, while differences in height were smaller (range 53–86 cm). Differences in plant spread were large (range of 107–226 cm) with plants in Groups IV and V having the largest spread and those in Groups 1 and II the lowest spread. Conversely, differences between groups in leaf width, leaf ratio, leaf sheath hairiness and inflorescence were small, while there were no differences between groups in terms of leafiness, leaf length, growth habit, culm thickness and stigma color.

Table 3. Eigenvector coefficients of 13 characters for the first 4 principal components with eigenvalue, individual and cumulative percentage of the total variance.

Character	Principal Component			
	First	Second	Third	Fourth
Days to 50% flowering	0.422	-0.117	-0.409	-0.106
Flowering duration	0.300	-0.036	-0.496	-0.306
Plant height	0.425	0.237	0.002	0.020
Leafiness	-0.006	0.118	0.254	-0.505
Growth habit	-0.018	0.234	-0.387	0.377
Culm thickness	0.182	0.006	0.265	0.627
Leaf length	0.178	0.546	0.030	0.029
Leaf width	0.432	0.028	0.341	-0.099
Leaf ratio	-0.208	0.419	-0.264	0.157
Leaf sheath hairiness	0.263	-0.284	0.223	0.149
Inflorescence width	0.097	0.467	0.224	-0.117
Stigma color	-0.027	0.290	0.119	-0.120
Plant spread	0.419	-0.053	-0.043	0.137
Eigenvalue	3.094	2.627	1.880	1.286
Individual percentage	23.80	20.21	14.46	9.88
Cumulative percentage	23.80	44.01	58.47	68.35

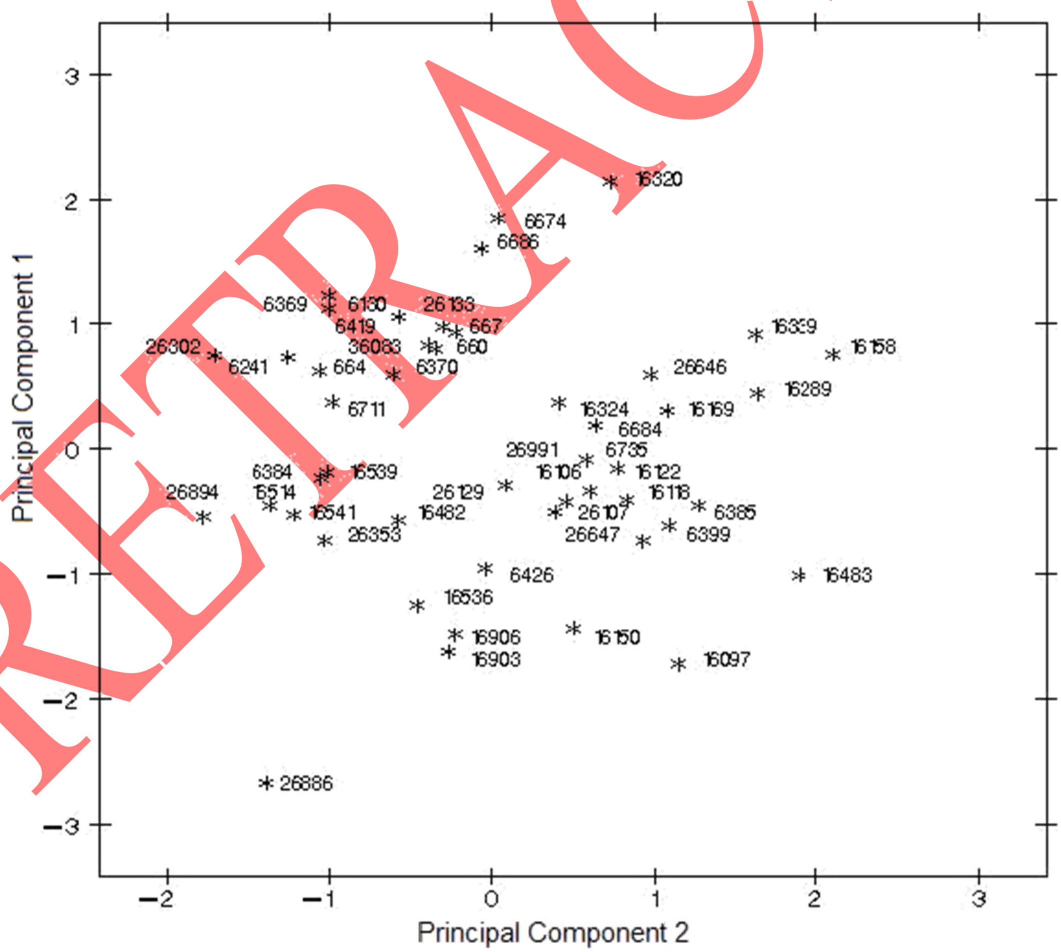

Figure 1. Scatter diagram of 47 *Urochloa* accessions plotted against the first 2 principal components of the correlation matrix explaining 44% of the total variation.

Table 4. Means of agro-morphological characters showing differences among clusters of 47 *Urochloa* accessions.

Character	Cluster group				
	1	II	III	IV	V
Number of accessions included	13	16	11	5	2
Time to 50% flowering (days)	129b[1]	91c	138b	127b	194a
Flowering duration (days)	49.0b	25.1c	59.1b	25.0c	104.0a
Plant height (cm)	68.0b	52.7b	85.6a	69.5ab	70.6ab
Leafiness	6.6a	7.0a	7.2a	6.6a	5.5a
Growth habit	3.7a	3.9a	3.9a	3.2a	6.5a
Culm thickness (cm)	2.9a	3.0a	3.6a	6.4a	2.9a
Leaf length (cm)	20.3a	21.7a	29.0a	16.8a	19.7a
Leaf width (mm)	13.1bc	10.4cd	14.7ab	15.0a	9.9d
Leaf ratio	15.8b	22.2a	21.3a	11.1b	20.6ab
Leaf sheath hairiness	2.3b	1.9b	2.5b	4.3a	1.6b
Inflorescence width (mm)	8.8b	10.2b	13.4a	8.5b	4.5b
Stigma color	3.2a	3.6a	3.9a	2.6a	2.0a
Plant spread (cm)	116c	107c	179b	226a	216a

[1]Within a row, means followed by different letters differ significantly at $P<0.05$.

Cluster analysis based on agro-morphological characters highlighted 5 main groups as shown in the dendrogram (Figure 2). The first level of separation (Group V vs. others) was mainly on the basis of days to 50% flowering and flowering duration. The 2 accessions classified in Group V, both *U. humidicola* (CIAT 6369 and 36083), were late-flowering (Table 4) and took 194 days to flower. While accession CIAT 36083 originated from Ethiopia, the origin of accession CIAT 6369 is unknown. The next separation (Groups IV and III) occurred due to differences in plant spread, leaf width, leaf ratio, leaf sheath hairiness, inflorescence width and plant height. Accessions in Group IV had greater spread, broader leaves and more hairiness, while accessions in Group III were taller and had greater inflorescence width. The 5 accessions in Group IV were evenly distributed, coming from Zaire, Kenya, Uganda, Rwanda and unknown origin and represented *U. ruziziensis* and *U. decumbens*. The 11 accessions in Group III were all *U. brizantha,* with most accessions from Ethiopia but some of unknown origin. Separation of Groups I and II was mainly on the basis of days to 50% flowering, flowering duration, leaf width and leaf ratio. The majority of accessions in Group II originated from Kenya, Ethiopia and Zimbabwe and represented 4 species: *U. brizantha, U. nigropedata* (Munro ex Ficalho & Hiern) A.M. Torres & C.M. Morton [syn. *B. nigropedata* (Munro ex Ficalho & Hiern) Stapf], *B. jubata* and *U. lata* (Schumach.) C.E. Hubb. [syn. *B. lata* (Schumach.) C.E. Hubb.]. Accessions in Group I were late-flowering and had broader and larger leaves than those in Group II, representing *U. brizantha, U. decumbens, U. ruziziensis, U. subquadripara* (Trin.) R.D. Webster (syn. *B. subquadripara* (Trin.) Hitchc., *B. jubata* and *U. lata,* with 5 of the 13 accessions coming from Kenya.

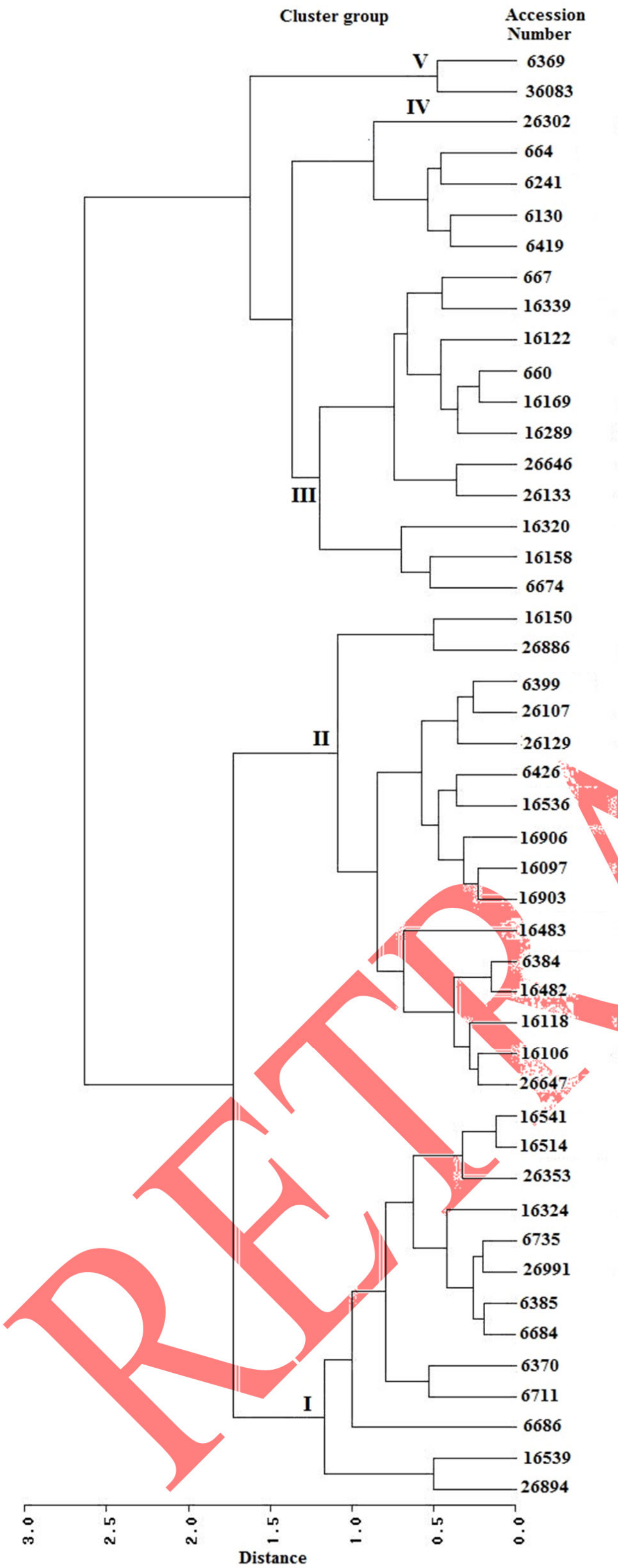

Figure 2. Dendrogram of agro-morphological classification of 47 *Urochloa* accessions obtained from complete clustering on 13 characters. Accession numbers belong to CIAT.

Discussion

Classification using the agro-morphological technique is useful in defining groups based on agronomic characters (Pengelly et al. 1992). In this study, 13 plant characters were selected from a total of 22 morphological and agronomic characters. The principal component and cluster analyses showed existing phenotypic variability among *Urochloa* species. Approximately 60% of the accessions evaluated were *U. brizantha* and the majority of these originated from Eastern Africa, which is regarded as the center of genetic diversity of *Urochloa*. Ethiopia and Kenya accounted for about 51% of the origins of the tested accessions. A good number of accessions were from Burundi, Rwanda and Zimbabwe, while accessions from Uganda, Zaire, Oman and Togo were poorly represented. Our classification into groups was not limited to one country with materials from the same region being classified in different groups indicating the degree of phenotypic diversity within the *Urochloa* genus. The 11 accessions which were collected from Ethiopia were distributed in 4 of the 5 clusters, while 13 accessions from Kenya belonged to 3 clusters. It is possible that different accessions which are phenotypically similar occur in different countries. However, genetically closely related accessions can have very different morphology and therefore different prospective use and agronomic value (van de Wouw et al. 1999a).

Agro-morphological characters were variable in determining composition of the groups with 50% flowering, flowering duration and plant spread being key determinants. These characters are important and form the basis of selection for different environments and forms of utilization. Flowering data are important adaptive characteristics (Hassen et al. 2006), with early flowering ensuring survival and sustainability in areas with a short growing period. On the other hand, accessions CIAT 6369 and 36083 that flowered late would be useful in areas with a long growing season. Those that have a wide spreading habit in cluster IV would be useful as ground cover to reduce soil erosion, while tall accessions which occurred in cluster III would be suitable for cut-and-carry livestock feeding systems.

The variance accounted for by the first and second components for agro-morphological data was 44%, a relatively low percentage of total variation compared with >75% obtained by Hassen et al. (2006) and Veasey et al. (2001). Normally variation of >75% is required to satisfactorily explain the variability expressed between individual accessions (Veasey et al. 2001). It is important to note that most of the accessions originated from only 8 countries in Africa and from each country only a few accessions were classified, so the material does not reflect the total *Urochloa* phenotypic diversity that exists within the region. Furthermore, even within the countries of origin the accessions were from similar agro-ecological zones. For example, all 13 accessions evaluated from Kenya were collected from within the Rift Valley region. This was a major limitation of our study as the material selected was not sufficiently diverse. There is a need to expand the collection to cover wider agro-ecological zones in addition to exploring germplasm from other countries. Nevertheless, our results provide useful information on the phenotypic diversity among the *Urochloa* accessions tested. This information adds to the pool of knowledge on collections of *Urochloa* species in Africa and would be useful in future evaluations. Attributes like dry matter yield and chemical composition need to be assessed on these accessions before proceeding with any further evaluation in the field.

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(Note of the editors: All hyperlinks were verified 14 January 2019.)

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Research Paper

Demonstrating control of forage allowance for beef cattle grazing Campos grassland in Uruguay to improve system productivity

Demostración de la mejora en la productividad de los sistemas ganaderos a través del control de la oferta de forraje sobre pasturas de Campos en Uruguay

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Abstract

While low-cost technology can be applied within beef cattle systems to improve economic output and decrease economic risk, methodologies to increase adoption by farmers deserve attention. Here we report 4 case studies where low-cost, high-impact technology was applied on commercial farms in an endeavor to demonstrate increased physical output in what we describe as 'Producer Demonstration Sites'. Forage allowance (FA) affects forage growth, forage intake by animals and energy partitioning to maintenance or production. We decided to demonstrate the benefits to production from controlling forage allowance at specific recommended levels. While we focused on FA, other management tools, e.g. suckling restriction and energy supplementation of cows prior to breeding, were tested in different contexts and time periods to improve the critical responses mentioned. While increases in production from 3 of the farms were demonstrated, only 2 of the farmers showed interest in implementing the strategies on their farms subsequently. We conclude that control of forage allowance improved energy intake and animal productivity. For this approach to be successful and increase adoption, it is important to involve the farmers in discussions regarding the proposed changes from the outset as well as the monitoring of progress during the demonstration.

Keywords: Animal management, cattle performance, research validation, spatial-temporal arrangement, stocking rate, subtropical pastures.

Resumen

Aunque existe tecnología de bajo costo para que los sistemas ganaderos incrementen su rentabilidad y disminuyan el riesgo económico, los métodos para incrementar su adopción por los ganaderos requieren mayor atención. Aquí reportamos cuatro casos de estudio donde tecnología de bajo costo y alto impacto fue aplicada en ganaderías comerciales para demostrar cómo incrementar la productividad física, en lo que llamamos 'Sitios Demostrativos'. La oferta de forraje (FA) afecta la producción y consumo de forraje por los animales así como la partición de los nutrientes hacia mantenimiento o producción. Decidimos demostrar los beneficios de controlar la FA en niveles específicos recomendados por resultados experimentales. Aunque nos enfocamos en la FA, otras herramientas, ej. restricción del amamantamiento y suplementación energética durante el entore, fueron recomendadas en diferentes contextos y momentos para mejorar la respuesta animal. Aunque demostramos incrementos en la productividad en tres de los cuatro casos, solamente dos ganaderos se mostraron interesados en continuar implementando las medidas subsecuentemente.

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Concluimos que el control de la FA mejoró el consumo de energía y la productividad animal. Para que este tipo de aproximación resulte en mayor adopción, es importante involucrar a los ganaderos en las discusiones sobre los cambios propuestos así como también en el monitoreo durante el progreso de la demostración.

Palabras clave: Arreglo espacio-temporal de la carga animal, manejo animal, pasturas subtropicales, desempeño animal, validación de tecnología.

Introduction

In Campos grassland (Allen et al. 2011) low net income of beef systems (Aguerre et al. 2015) and degradation of natural grassland by over- or under-stocking are major problems confronting livestock farmers. Beef grazing systems are subject to high variability in herbage production between and within seasons primarily because of rainfall and temperature variability and animal energy requirements due to physiological stage in breeding females (lactation, gestation, mating) or stage of growth in fattening cattle. However, manipulation of forage allowance [FA, in kg DM/kg live weight (LW); Sollenberger et al. 2005] coupled with suckling restriction, flushing and weaning for cow-calf systems and sown perennial pastures with grazing-time restriction and allocation for fattening systems could reduce the impact of variability in the feed resource on animal production. Management of FA requires variation of stocking rate ('put-and-take' method) at paddock or system scale, and measurement of forage mass. Previous authors have found that criteria for variation of stocking rates on farms normally are not based on experimental information, which may reduce the opportunity to improve animal production (Paparamborda 2017). Forage allowance (FA) experiments demonstrated the benefits of its control, improving animal production per ha in beef cow-calf systems by increasing individual animal production at lower or equal stocking rates (Claramunt et al. 2017; Do Carmo et al. 2018).

To control FA from paddock to paddock through time, stocking rates have to be varied. In years of below-average rainfall, areas of stockpiled forage may be needed to maintain stocking rates (Derner and Augustine 2016), and/or supplementation in combination with stockpiled forage may be adopted, because sales are not possible without adverse economic consequences (Derner and Augustine 2016). The farm area allocated for forage conservation will depend on the potential accumulation of forage per ha, the period without forage production owing to dry or cold conditions when feeding is needed, the number of animals to be retained during the critical period (essential nucleus of the system, sales planning) and average stocking rates achieved under different FA levels for many years. Given the variability in forage production between years, maintenance of a more or less equal number of livestock and level of production can be achieved only with stockpiled forage (which leads to moderate stocking rates at the system scale) and/or supplementation (with concentrates) during the critical years. Feeding of stockpiled forage can be combined with nitrogen fertilization of native pasture (C4 plants, to increase herbage accumulation) in spring in areas where high responses to N fertilizer are achieved due to deep soils with good water availability (Derner and Augustine 2016).

For beef cow-calf systems, Soca et al. (2007) and Do Carmo et al. (2016) at the School of Agronomy, Universidad de la República (Uruguay) proposed that the breeding and calving seasons and hence cow energy requirements be synchronized with the pattern of forage production (Funston et al. 2016). This would also ensure satisfactory body condition scores (BCS) at calving. They recommended that the breeding season commence at the beginning of summer (December) to achieve high annual pregnancy rates. Short-term suckling restriction and energy supplementation at the beginning of the breeding season were recommended for primiparous and multiparous cows calving in lower BCS than recommended (Soca et al. 2013; Do Carmo et al. 2016).

For growing beef steers and heifers, grazing experiments in Brazil on Campos grassland showed the benefits on animal productivity per animal and per ha of controlling FA, although with high variability in average daily gain (ADG) (Soares et al. 2005; Mezzalira et al. 2012). When FA was changed for different seasons, i.e. 1 kg DM/kg LW in spring and 2–3 kg DM/kg LW in summer, autumn and winter, ADG during winter was improved (Soares et al. 2005). On farms, the farmer has the opportunity to vary the combinations of paddocks used, the timing of grazing, the classes of stock grazing at particular times and the actual FAs for different times and groups. For instance, beef cows can be used during winter (FA ≥3 kg DM/kg LW) to remove the mature dry forage, improving the pasture condition for growing-fattening steers during the next growing season. Monitoring of forage mass over time within and between paddocks is imperative for managers to assign appropriate stocking rates and animal categories based on pasture condition and FA standards. This allows for better decision making for each animal category and aids in coping with climate variability (Derner and Augustine 2016).

Three factors influence the production from grazing systems, namely: forage growth; forage intake; and energy partitioning to animal maintenance or production; and the 3 processes can be improved by controlling FA at a high level (Moojen and Maraschin 2002; Do Carmo et al. 2016). For this reason we focused primarily on managing FA, and applied the other tools (suckling restriction, nutritional flushing of breeding females, grazing time restriction and grazing sown pasture) when appropriate.

Our hypothesis was that manipulation of FA in any beef production system (cow-calf, growing or fattening) could improve production from the particular system, and also indirectly improve the other processes and positively affect the overall farming system. For this reason we focused on the most limiting process for each farm. Our objective was to demonstrate, within a technology transfer project, the benefits of manipulating FA in both cow-calf systems in association with other techniques (suckling restriction and flushing) and growing-fattening systems (joined with sown pastures and grazing time restriction). A further aim was to demonstrate the benefits of spatial-temporal management of paddocks by grazing with growing animals (spring–autumn, i.e. September–May) and beef cows (winter, i.e. June–August) through improved forage use efficiency. We aimed to increase acceptance of these strategies by demonstrating them on 4 commercial beef grazing systems as 'Producer Demonstration Sites'. In selecting the particular sites we considered the unique characteristics, restrictions and knowledge of each manager and employed a continuous dialogue in implementing the techniques proposed, in an easy way according to each manager.

Materials and Methods

Background

Technological proposals that focus on FA and its manipulation to improve animal production are available for growing cattle. When FA on Campos grassland was maintained between 2 and 3 kg DM/kg LW, ADG of growing steers varied from 0.25 to 0.5 kg/head/d on average, with annual gain per head varying between 91 and 180 kg (Moojen and Maraschin 2002; Soares et al. 2005; Mezzalira et al. 2012).

Forage allowance expressed as kg DM/kg LW has not been the most frequent expression of FA in Campos grassland experiments (Moojen and Maraschin 2002; Soares et al. 2005; Mezzalira et al. 2012). Rather, those works report FA as % of LW which require a time period specification, and affect the calculated value of stocking rate, although FA expressed as kg DM/kg LW is easy to explain and estimate, if data for forage mass (kg DM/ha) and stocking rate (in kg LW/ha) are available. We collected data for forage mass, stocking rate and ADG of growing steers grazing Campos grassland from published papers as well as M.Sc. and Ph.D. theses (Grazing Ecology Research Group at UFRGS, Brazil) to develop 'rules of thumb' for FA, which would provide an optimal combination of ADG and stocking rate (Table 1). For beef cows, we developed FA standards based on research developed from 2007 (Do Carmo et al. 2016; 2018) plus previous information that recommended target forage height and BCS of cows during the lactation-gestation cycle (Soca et al. 2007; see Table 1). For fattening animals we were unable to locate grazing experiments for Campos grassland, and for this reason standards presented are a mixture of beef cow values and empirical experience collected in this project (Table 1).

Table 1. Forage allowance (kg DM/kg LW) standards derived from research results which might apply in commercial systems.

Animal category	Forage allowance per season			
	Spring[1]	Summer	Autumn	Winter
Growing steers or heifers (up to 300 kg LW)	1–2	2–3	2–3	2–3
Beef cows	≥6	≥6	≥6	3–4
Fattening steers (300–500 kg LW)	4–6	4–6	4–6	10

[1]Seasons were defined as: Spring = September, October and November; Summer = December, January and February; Autumn = March, April and May; Winter = June, July and August.

Producer Demonstration Sites and methodological procedures

From May to September of 2012, we spent time looking for 'partners' inside groups of farmers. Finally, we chose 4 farms, each one inside of one group of farmers as follows: Case 1 located at 31°35´ S, 56°30´ W; Case 2 located at 32°32´ S, 56°07´ W; Case 3 located at 32°34´ S, 54°40´ W; and Case 4 located at 33°28´ S, 55°26´ W. The study was conducted from November 2012 to July 2015. Rainfall on the farms or nearby is presented in Figure 1, and soil types and botanical composition of pastures on each farm are presented in Table 2.

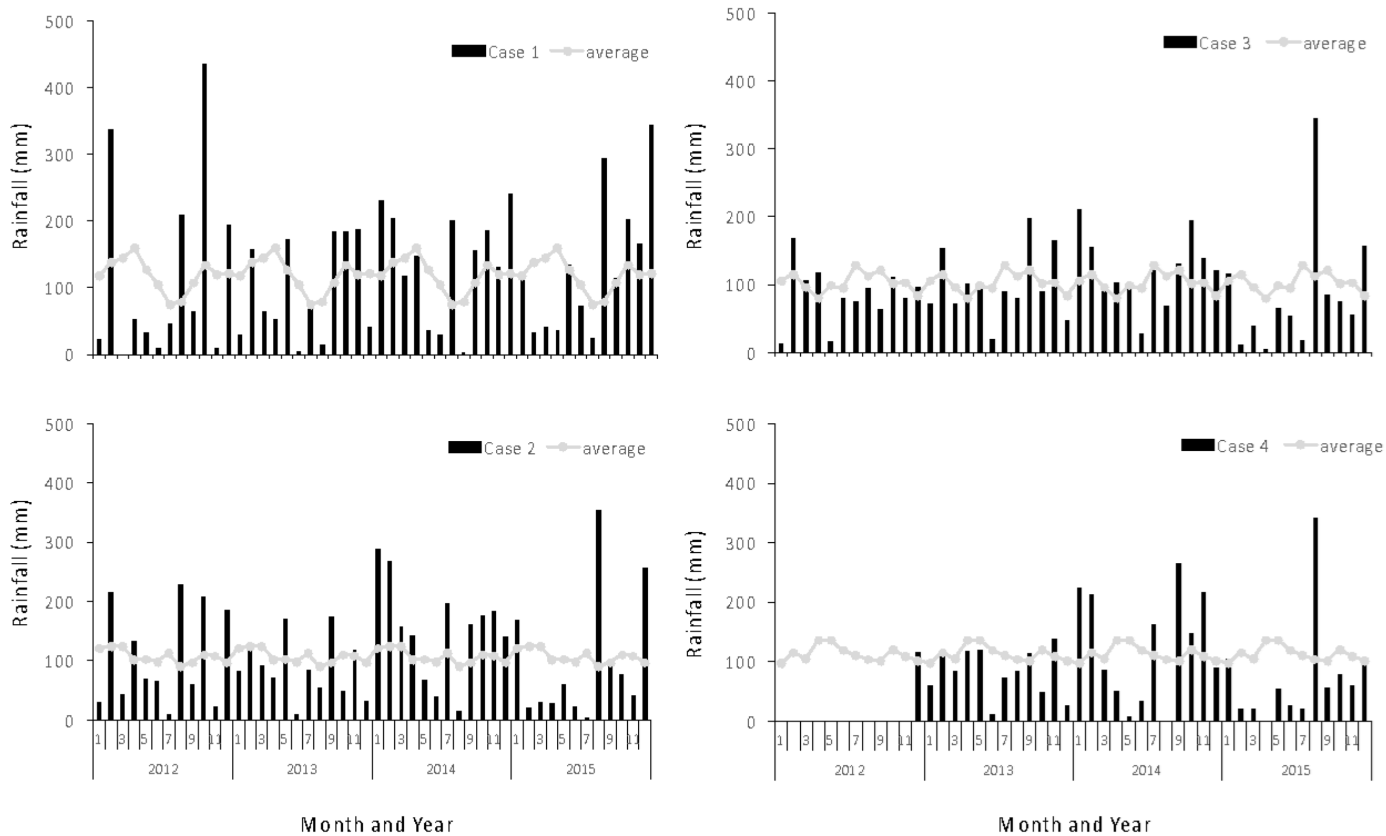

Figure 1. Monthly rainfall (2012–2015) near the farm (Cases 1 and 2) and on the farm (Cases 3 and 4) compared with the medium-term average (1961–1990) for the nearest city to each farm. Rainfall data for Case 4 during 2012 were not available.

Table 2. Soil and vegetation characteristics where 'Producer Demonstration Sites' were established.

	Soils[1] (USDA classification)	Vegetation (% of each species)
Case 1	Udorthents and rock outcrops (mainly); Hapludolls in minor proportion	*Paspalum notatum* (17%), *Bothriochloa laguroides* (14%), *Schizachyrium spicatum* (10%), *Aristida echinulata* and *Piptochaetium montevidense* (5% each), *Coelorachis selloana* and *Cyperus* sp. (4% each)
Case 2	Hapludolls, Argiudolls and Hapluderts (mainly) and Udorthents and rock outcrops (lower proportion)	*Paspalum notatum* (25%), *Bothriochloa laguroides* (12%), *Schizachyrium spicatum* (10%), *Coelorachis selloana* and *Trachypogon montufari* (7% each), *Aristida venustula* and *Cyperus* sp. (6% each)
Case 3	Dystrudepts and rock outcrops	*Paspalum notatum* (28%), *Cynodon dactylon* (10%), *Axonopus* sp. and *Paspalum plicatulum* (8% each), *Piptochaetium montevidense* and *Setaria* sp. (5% each)
Case 4	Argiudolls, Hapludolls, Hapludalfs and rock outcrops	*Axonopus* sp. and *Paspalum notatum* (20% each), *Bothriochloa laguroides*, *Cyperus* sp., *Paspalum dilatatum* and *Coelorachis selloana* (5% each)

[1]Soil information is based on Durán et al. (1999).

For cow-calf systems, manipulation of FA was coupled with suckling restriction (calves heavier than 70 kg fitted with nose plates for 11 days starting at the beginning of the breeding season) and flushing of cows (2 kg of rice bran, on a fresh basis, for 20–25 days, after suckling restriction; Soca et al. 2013) at the beginning of the breeding season and weaning of calves in March-April. Suckling restriction was applied only to calves heavier than 70 kg (Soca et al. 2007) and all calves received suckling restriction at different times (in Case 3 in 2 groups, based on weight of calves).

For fattening steers, even when very high FA on native pastures was applied during autumn-winter, loss of live weight was observed. In order to overcome this phenomenon, we proposed grazing pastures of ryegrass (*Lolium* spp.) or fescue (*Festuca* spp.) during this period (fescue is a perennial grass and is the cheaper option, but has lower initial forage production), since they produce high quality forage during the autumn-winter period. Restriction of grazing time has the potential to increase carrying capacity of the pasture under use (Soca et al. 2010), and introducing animals to the pastures at dusk has the potential to improve forage utilization (Gregorini et al. 2006). For these reasons, we promoted restricted grazing time (18:00–6:00 h), to achieve high stocking rates on the ryegrass without affecting animal performance (Gregorini et al. 2006).

On the other hand, to alleviate climatic variability in grasslands with shallow soils where conditions for forage production are 'narrow' (high risk of drought), applying nitrogen fertilizer to native pastures during spring and summer (growing season) can greatly contribute to improved forage production and we recommended it in one case (Case 1).

In 'real systems' the concepts exposed above are often not applied, possibly due to ignorance or lack of 'know how' to apply appropriate management options, perceptions of greater effort involved or that the extra income does not justify the effort involved.

Measurements of forage mass and height were obtained by the 'comparative yield method' (Haydock and Shaw 1975) using a 5 or more points scale (depending on the range of heterogeneity of the herbage), with samples spanning the range being harvested and dried in a forced-air oven at 60 °C until constant weight. Using data from the harvested samples as reference points, a systematic sampling procedure (>100 points per paddock) was used to visually estimate the average forage mass. Forage height was measured with a ruler (in the 5 point scale quadrats) at the level below which 80% of the vegetation was estimated to occur visually, ignoring tall stems (Stewart et al. 2001).

Body condition score (BCS) for beef cows was estimated visually using the system of Vizcarra et al. (1986), and unfasted live weights of animals (LW) were used.

In Table 3 general information on the 4 farms at the beginning of the project is presented.

Case 1. As shown in Table 3, productive performance was already high, i.e. pregnancy rate was high and age of steers at slaughter indicated that ADGs would be in the range of experimental reports for growing steers and heifers (as explained above). However, the objective was to increase output of fattening steers, and we saw an opportunity to improve fattening by altering the system of paddock use by the various animal categories. Some paddocks were traditionally assigned to a particular animal category, e.g. 1 paddock was used exclusively by steers, because it was considered a 'good' paddock given the combination of deep (30–40%) and shallow (70–60%) soils, and water availability (water from a stream across the paddock). We proposed to graze this area with steers only during spring-autumn and introduce pregnant beef cows during winter, in order to remove the mature dry forage accumulated during the spring-autumn period. Forage allowance during winter was monitored monthly and kept at 3–4 kg DM/kg LW (Do Carmo et al. 2018), which is a low FA for beef cows. High stocking pressure is needed to adjust the coefficient following a growing season when FA was high, e.g. if herbage mass is 2,500 kg DM/ha and desired FA is 3 kg DM/kg LW, then 833 kg LW/ha should be placed in the paddock. At the end of winter, mature forage was removed by the cows, herbage mass being decreased from 1,852±1,118 kg DM/ha (average ± standard deviation) at the end of autumn to 994±923 kg DM/ha at the end of winter and a new cycle of steer fattening commenced, with FA adjusted to ≥6 kg DM/kg LW. In previous years, steers lost weight in this paddock during winter; with the rearrangement, shifting steers to other paddocks, which were grazed until the end of summer and destocked to accumulate forage during autumn, allowed steers to either maintain or lose less weight during winter. Experimentally we used this protocol of lower FA during winter to consume the forage generated during the growing season, with an increment in the stocking rate (lower FA) of the paddock (Do Carmo et al. 2018).

As a further measure, owing to the high percentage of shallow soils and climatic variability, we encouraged the manager to increase forage production by applying N fertilizer to the paddock (22 ha) with the deepest soils and the greatest water storage capacity. This strategy was designed to reduce the effects of rainfall variability on livestock energy intake, and ensure forage was available in the event of severe climatic conditions. Greater herbage production without increasing stocking rate can result in increases in individual forage intake or stockpiled forage.

Other management technologies suggested, like levels of FA or suckling restriction, were already being applied by the farmer. The FA proposed for steers and beef cows (Table 1) was similar to that employed by the manager from his own experience. Production from the farm in kg LW (sales – purchases – deaths +/- differences in total weight of animals during a year; i.e. 100–110 kg LW/ha annually) was relatively high compared with the national average, i.e. 64% weaning rate and 70 kg LW/ha per year (Paparamborda 2017).

Table 3. General information for each farm and each beef process at the beginning of the technology transfer project.

	Case 1	Case 2	Case 3	Case 4
Farm size (ha)	450	2,200	260	360
Average stocking pressure (kg LW/ha)	230–260	300–320	250	230–320
Historic pregnancy rate (%)	85–95	85–95	70	---
Season of breeding	Summer	Summer	Summer and Autumn	---
Age of steers at slaughter (years)	3–3.5	3–3.5	---	---
Age of heifers at first joining	2	2	2–3	---
Forage mass (kg DM/ha) at the beginning	1,600	6,000	978	3,000
No. of breeding cows	80	200	94	---
No. of steers	129	800–900	---	270

Case 2. On this farm all animal processes were practiced, e.g. breeding cows and growing and fattening steers, and production was near 140 kg LW/cow/year, suggested as the desirable goal (Moojen and Maraschin 2002; Soares et al. 2005; Mezzalira et al. 2012). Since pregnancy rates in the cow-calf operation were already high (Table 3) and we observed that forage mass remaining on pastures at the end of grazing by steers was higher (6,000 kg DM/ha) than was needed (2,500–3,000 kg DM/ha), we focused on refining the growing and fattening process. Our aim was to increase stocking pressure and have steers consume a greater percentage of available pasture, thereby increasing production per ha, but without changing liveweight gains per animal.

As in Case 1, paddocks were 'historically' assigned to different categories of animal, with paddocks containing deepest soils and probably greater forage production being assigned to steers all-year-round. In the first year FA for fattening steers (>400 kg LW) was increased from 5 to 8 kg DM/kg LW depending on ADGs during the previous month for spring-autumn, and during winter it was further increased to 10 kg DM/kg LW. However, ADGs were negative from May to August (autumn-winter) during the first year, so we proposed introducing 2 changes. The first was to graze beef cows on these areas during winter to remove the mature dry forage accumulated during the previous growing season when grazed by fattening steers (as in Case 1). Secondly, to overcome the problem of negative ADGs during winter with heavy steers we considered 2 possible options: the feeding of supplements or planting C3 pastures like ryegrass or fescue. We encouraged the manager to plant perennial ryegrass (first year), that behaves as an annual ryegrass without seed production, with the aim of planting perennial fescue pasture the following year in order to reduce the unit cost of the DM produced. During 2014, 40 ha were planted with ryegrass, and steers were allowed access to this pasture only from 18:00 h to 6:00 h as proposed by Gregorini et al. (2006) and Soca et al. (2010). During the day they returned to native pastures (stocking rate of 4 steers/ha, FA of 0.8–1.5 kg DM/kg LW) with water ad libitum. Stocking pressure on ryegrass was regulated via a 'put-and-take' method, to allow 2 or 3 kg DM/kg LW and/or 6–10 cm residual forage height.

Based on information from Soares et al. (2005) and Trindade et al. (2016) and the expectation of better performance when forage mass was in the range of 1,500–2,000 kg DM/ha, we allocated growing steers and heifers to paddocks with a higher percentage of shallow soils, where forage growth was lower and it was easier to maintain forage mass in this range. However, these paddocks were larger than 100 ha and distance from water points to some sections of the paddock exceeded 1 km, which can affect grazing distribution negatively. To make grazing distribution more even we encouraged farmers to drive their stock to distant grass areas and to place low-moisture protein supplements on these distant areas (Bailey et al. 2008).

Case 3. This farm occupied 860 ha divided into 3 separate 'fields', 2 of which were mostly grazed by livestock not owned by the land owner, so we focused on the 'field' (260 ha) grazed by his own livestock. The main activity there was a cow-calf operation. While previous records of calving and weaning rates and weights of calves were limited and it was not possible to reconstruct them, the owner claimed a weaning rate around 70%. The owner's first restriction on management changes was that total number of animals on the area could not be changed, but internal movement between paddocks within the 'field' was possible. The 'field' was comprised of 4 paddocks: 160 (Main), 80, 14 and 4 ha. The last 2 paddocks contained native pastures oversown with *Lotus subbiflorus* cv. El Rincón, and were used to fatten culled cows. We made all the measurements of forage on the Main paddock and focused on lactating or pregnant cows, the most energy-demanding category.

First, we measured forage mass (978±354 kg DM/ha) and cow BCS (4.4±0.63) in November 2012 (spring-summer; Figure 2). The herd in the Main paddock contained lactating cows, early pregnant cows, growing heifers and cows that had not calved the previous year (it is a common practice to maintain non-pregnant cows from one year to the next, even though they eat grass without producing calves). Target FA was 6 kg DM/kg LW in the Main paddock, to increase forage production and energy intake of lactating cows. At the same time, for lactating cows with calves heavier than 70 kg, suckling restriction (attaching a nose plate to the calf to prevent suckling) was applied for 11 days (Soca et al. 2013; Figure 2). To reach the desired FA of 6 kg DM/kg LW in the Main paddock, we relocated some animals based on physiological status (lactating or empty or pregnant) and energy stored in the body (body condition score). Pregnant cows, growing heifers, non-gestating non-lactating cows (empty dry cows) and cows with BCS greater than 4.5 (n=24 of 96 cows) and a bull were removed to the 80 ha paddock at the beginning of the breeding season (10 December 2012). Animals moved to this smaller paddock had lower energy requirements than lactating cows with BCS lower than 4.5. Forage allowance in the Main paddock in November 2012 was 4.4 kg DM/kg LW, which increased to 6.2 kg DM/kg LW after relocation of the less-demanding animals. This increment in forage allowance resulted in an increase in forage mass in the following month (1,272±755 kg DM/ha), helped by the average rainy spring-summer of this year (Figure 1). Thus, to maintain FA at the desired level, some animals had to be re-introduced from the 80 ha paddock.

Forage mass increased until March 2013 (2,398±781 kg DM/ha) because of high forage growth (average rainy summer), and the FA was maintained at 6 kg DM/kg LW until May 2013. During winter, C4 plants grew little, and we adjusted numbers to maintain FA above 3 kg DM/kg LW. We recommended feeding cows a rice bran supplement at the beginning of the breeding season in December-January (after suckling restriction period); however, this advice was not heeded.

During winter 2013, sheep (n=140) were introduced, in part to control weeds (*Senecio selloi* and *S. grisebachii*), and were maintained throughout the project period.

Case 4. This farm was composed of 5 separate 'fields', totaling 2,362 ha. We worked on a single paddock of 120 ha within a 'field' of 360 ha, and growing beef steers was the main enterprise. The owner insisted that the herd be managed as a whole, so we could not move a group of animals to other paddocks within the 'field'. This inflexible restriction virtually made all efforts futile. To control FA (relationship between forage mass and animal live weight per unit area), herd size or area of the paddock must be modified when changes in forage mass occur. In both years we adjusted stocking rate in May to provide FA of 6 kg DM/kg LW, which is not the best FA for growing animals (Table 1). Employing a lower level of FA may result in overgrazing for an extended period and make later adjustments impossible. After the FA adjustment in May, FA was monitored without the possibility of varying animal number.

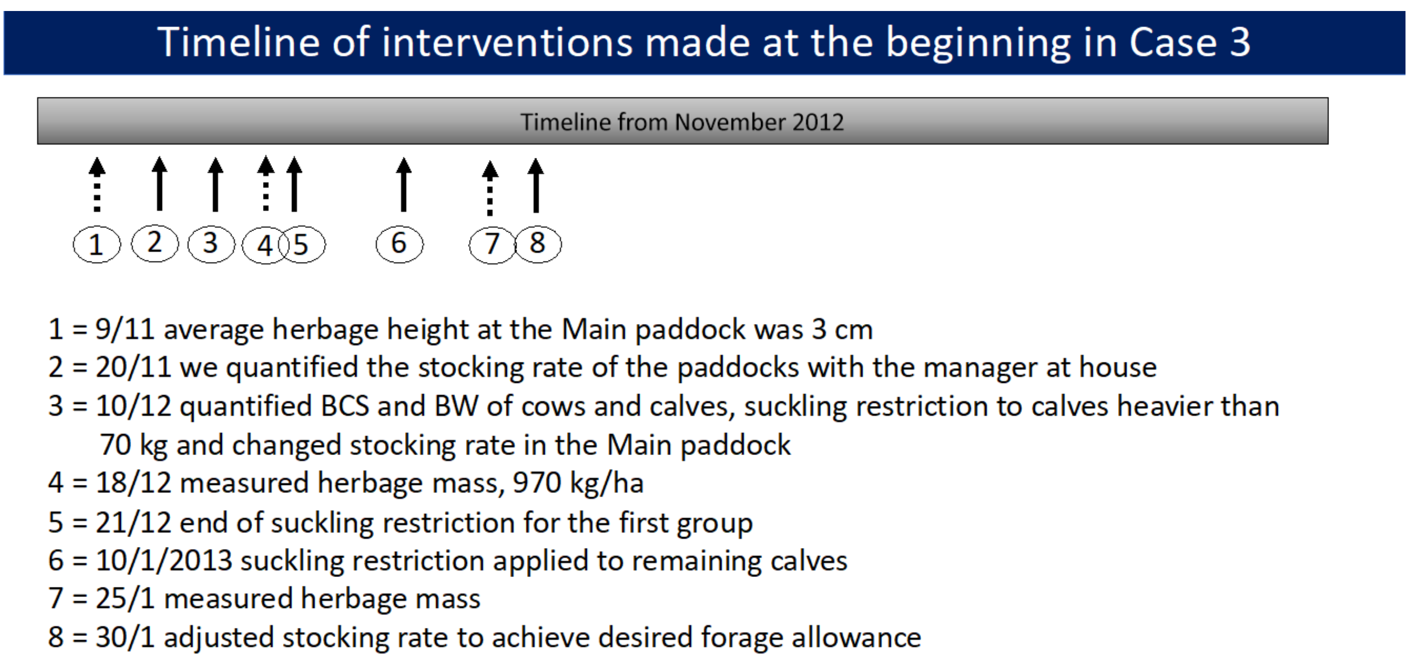

Figure 2. Sequence of tasks and decision-making activities at the beginning of the breeding season for Case 3. Dotted arrow represents forage measurements and solid arrow task or decision taken. Dates are expressed as day/month/year.

Results

Case 1

Since original management of the cow-calf and fattening systems approached the recommended strategies, increments in production were marginal, i.e. 8–9%, an increase of 10 kg LW/ha/yr from a base of 110 kg LW/ha. Changes of paddock and seasonal use by different animal categories plus applying nitrogen fertilizer to pasture were the innovations applied, as FAs already employed by the producer were close to recommended levels and were maintained. Suckling restriction (use of nose plates on calves), already a current practice on the farm, was applied along with feeding an energy supplement to flush cows before the breeding season during a short drought (December 2013 and 2014).

Case 2

On native pastures, positive ADGs ranged from 0.1 to 1.4 kg/d, depending on season and paddock, but our data recording did not permit us to determine if LW gain/year was improved, because we focused on finishing of heavier steers and not recording LW changes for single groups of animals throughout a complete year. Stocking pressure in paddocks under FA control during the fattening phase was 560±297 kg LW/ha, while the average on the farm was 342 kg LW/ha. Increments in LW production were 63 and 21% from the average of 120 kg LW/ha/yr for Years 1 and 2 at the paddock scale. Seasons greatly affected ADGs, which were -0.42, 0.9, 0.55 and 0.03 kg/hd/d during Winter, Spring, Summer and Autumn, respectively, for fattening animals, although these are not for the same animals in different seasons. For growing animals ADGs were -0.15, 0.5, 0.5 and 0.08 kg/hd/d during Winter, Spring, Summer and Autumn, respectively. On ryegrass pastures ADGs ranged from 0.8 to 1.1 kg/hd/d and stocking pressure from 825 to 1,487 kg LW/ha during 2014 and 2015. However during 2015 the growing season for ryegrass was limited by severe drought conditions (Figure 1) during Autumn-Winter 2015 that delayed the establishment and growth of the pasture.

Case 3

Mean calf weight at weaning in May was 153 kg with pregnancy rate (April) of 88%. For the second year, pregnancy rate was 90% and calf weight at weaning (April) was 168 kg. Pregnancy rates for Years 1 and 2 were 7 and 18% units, respectively, greater on the focus farm than on a large sample of farms in the area (47,000 cows analyzed). Stocking pressure (255±61 kg LW/ha) was maintained throughout the years, with any variation imposed by the manager and FA management. In Figure 3 we show the stocking pressure management within the Main paddock and the rest of the farm (and average of the farm) to adjust FAs to our recommendations. It shows the 'put-and-take' method on the farm as a whole, where stock numbers in the Main paddock and the rest of the farm were modified to maintain desired FAs. First, stocking pressure increased on the rest of the farm, although as forage mass increased in the Main paddock, stock were reintroduced to increase stocking pressure to maintain the desirable FA. The 'put-and-take' method was implemented taking into account physiological state and animal category, considering the energy demand or capacity to use body reserves. The product of weaning rate × calf LW at weaning represents the production of calf LW per cow exposed and values were 107, 135 and 151 kg LW/ exposed cow, for the 'historical', Year 1 and Year 2, respectively.

Case 4

Despite the severe restrictions to desirable management, production was 120 kg LW/ha in this paddock, 20 kg above the average for the other 'fields'.

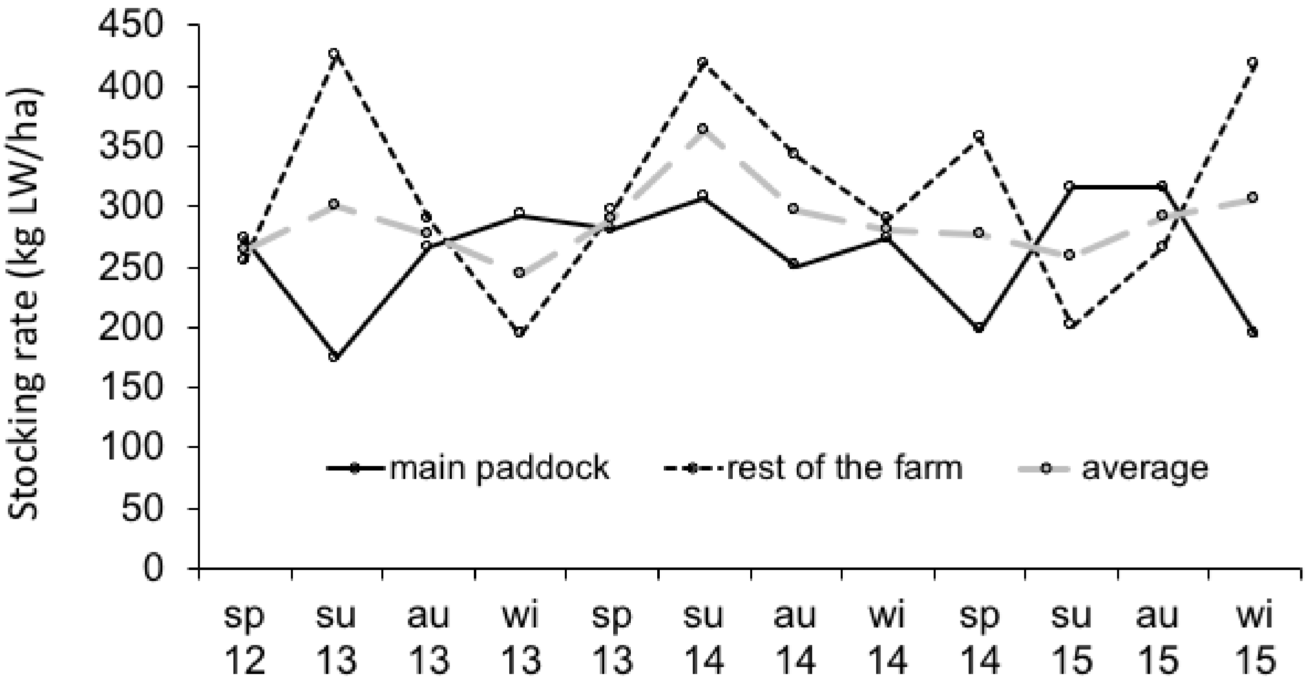

Figure 3. Stocking pressures (kg LW/ha) in the Main paddock and the rest of the farm (rest of property). Sp = spring, su = summer, au = autumn and wi = winter; numbers 12 to 15 represent the years 2012 to 2015.

Discussion

For selection of the co-operating producers, we met many groups of producers to explain our ideas and try to encourage them to follow the recommended process on the demonstration site. However we clearly failed to engage the groups of producers effectively and 2 of those who agreed to work with us (Cases 3 and 4) failed to accept our recommended management strategy.

Interaction with farm managers was challenging because their definition of success affected their willingness to implement change. Most of the recommended changes involved low-cost methods, which were easy to implement and directly impacted animal production, taking into account the 'constraints' of the farm or farmer. Animal production increased on all farms, but with differences due to plasticity-adaptability of the decision making of the farmer to change management. Economic output was not measured, but should have increased, because increments in animal production were achieved without greater cost (with the exception of sowing ryegrass in Farm 2 and applying N fertilizer in Farm 1, the other management changes do not impose additional costs and could decrease work load; Albicette et al. 2017), but greater decision-making was involved, addressing issues of time and space and different animal categories and physiological stages.

Case 1

Probably the most important change in the farm operations was the change in paddock use in winter by different animal categories, which allowed better individual performance for fattening steers, since previously they grazed the senescent herbage from the previous growing season resulting in weight loss. Whereas the reproductive performance of beef cows grazing on senescent herbage during gestation period is not affected, in spite of a reduction of their BCS from 5 or plus 5 to 4 or plus 4 (Do Carmo et al. 2016), the performance of steers grazing in another paddock on forage accumulated during autumn improves. Most of the other low-cost technologies were being applied on the farm before our intervention in the decision-making process. For the manager, adjusting stocking pressure to accommodate differences in available pasture was easy to apply because he had already done so on the farm, with quite good results in terms of increased animal production. However, the results and information obtained would have been enhanced if there was better trust between the parties; trust can be built up only over time, and the project term was short, which did not allow the development of the necessary trust to demand higher quantification of the process and outputs. The manager was kind, but a reserved person, and we were interested in: maintaining the relationship with him and his group; and making progress in terms of management of herbage and livestock, even with low registry of stocking rate and LW to make the process easy for the manager. The group of producers which he represented did not get involved with the management of the farm during the study, and did not participate in the discussions between us and the farmer on the farm. We have no information on whether they adopted any of the strategies subsequently.

Case 2

We started the project as a contest with the manager, as we managed 1 paddock (61 ha) and he managed the adjacent paddock (51 ha); the commitment was to not reduce ADG, but stocking rate could be changed according to our criteria. After 2 months of equal ADGs but greater stocking rates in the paddock under our control because of high forage mass (it was the opposite situation to Case 3, where we had to decrease the stocking rate in the Main paddock at the beginning), the manager decided to change the grazing management on the farm, and we were able to control the management of a larger area, up to 350 ha (5 paddocks). For native pastures, previous information about ADGs during fattening was unavailable, but Soares et al. (2005) had shown that ADGs for growing steers were greater during spring (0.8–1.4 kg/d) and summer (0.4–0.8 kg/d) than during autumn (-0.1 to +0.1 kg/d) and winter (-0.2 to -0.6 kg/d). Mezzalira et al. (2012) reported much lower ADGs on a similar experimental field (managed for more than 30 years under the same FAs), while Soares et al. (2005) reported on the same study and showed significant 'year' effects on ADGs under the same FA treatments. However other factors could be involved, e.g. paddock size in the experiments (Soares et al. 2005; Mezzalira et al. 2012) was lower than 10 ha while paddocks on the farm were from 50 to more than 100 ha. In the larger paddocks distance to watering points exceeded 1 km in some sections, which could result in uneven grazing and lower FAs on areas grazed. On this farm, stocking rate in the paddocks under our control had to be increased from the beginning, because forage mass was very high, and we had the opportunity to increase stocking pressure while maintaining ADGs, as a result of adjusting FA.

Paddock size affected the ability to graze a particular paddock with different animal categories (cows vs. steers) in different seasons (growing season and winter), because cow numbers were too low to apply this management in paddocks of 120 ha or greater. Therefore we used the cow herd in paddocks of 40–60 ha, to remove mature dry forage in winter. Adjusting animal categories grazing a given area allowed higher stocking pressures to be maintained than in paddocks grazed only with growing animals throughout (560 vs. 345 kg LW/ha).

At a system level, potential changes in overall production were not achieved because it was not possible to increase the total number of animals on the farm. For many periods of time more than 800 ha (of the total 2,200 ha) were ungrazed, showing the potential to increase the overall stock numbers without decreasing individual performance and as a consequence, increasing overall production. The management of FA, incorporating paddock condition-animal category or paddock condition-animal physiological state combinations, was performed on only 16% (350 from 2,200 ha) of the farm, and could be extended to the remainder to further improve the efficiency of forage utilization, as in Case 3. The 'put-and-take' method was applied before we arrived, based on the farm manager´s own criteria and not on experimental evidence. He became highly involved with the forage mass measurements and application of FA standards for management (Table 1), showing a great willingness to apply these principles and extend them to the other 'field' of 900 ha. A constraint on the area to which FA management can be applied is the capacity to perform estimates of forage mass in one day, timing for trips from and to the experimental station, and also the amount of forage mass samples to process and store. However the manager had found a way to apply the FA management by using paddocks, where the herbage mass was quantified, as a 'reference' to adjust FA in other paddocks without herbage mass quantification. His group of producers were more engaged in the management discussions which were held, although we do not know if it resulted in changes in management on their farms in terms of FAs and subsequent changes in production.

Case 3

Increments in pregnancy rate and calf weight at weaning were a product of FA management, herbage use efficiency matching energy requirements with FA and pasture condition and suckling restriction. Production of calf LW/cow exposed to bulls increased by 27–55% if one adopts a baseline pregnancy rate of 70% and calf weaning weight from the first year of 150 kg. Ruggia et al. (2015) and Tittonell et al. (2016) reported average increments of 20% for many farms employing the same approach (management and decision making changes with no input increments). In this case, the manager did not get involved with the measurements of forage mass and livestock management, which is the central issue of the strategy; he left all decision making in our hands, with the sole restriction of not changing the overall stock numbers. Although techniques were not completely applied, as we recommended, the impact on animal production was high. The failure of the manager to become fully involved with the demonstration was probably partially our fault, because communication was less than ideal. The benefits of the management changes were not well communicated, in the sense that the overall work load related to monitoring the health and nutritional status of the animals has the potential to decrease if planning and quantitative information (forage mass, cow BCS, FA management) are taken into account for decision making (Albicette et al. 2017). Despite increases in production the farmer was unwilling to change his existing management strategy and would have reverted to his old system when the study concluded. This illustrates the complexity of the process to convince farmers to alter their decision making processes, which may have been practiced for decades, even when economic benefits can be demonstrated.

Case 4

The inflexible management kept ADGs and production levels almost constant compared with the average, and at values lower than potentially achievable if flexible management could be applied (Soares et al. 2005). Owing to constraints imposed by the manager (fixed stocking rate or management of steers as a single group) the FA levels applied were higher than the recommended levels, as insurance against possible gaps between forage production and forage demand from animals, limiting potential improvement in meat production.

The manager considered that the proposed decision making process was highly complex or not compatible with his livestock management. This attitude is unfortunate as increases in animal production and economic outcome rely on improved management of FA coupled with other techniques that allow increased energy intake by growing animals.

Our commitment with his group of farmers, combined with the desire to change his mind about FA management, kept us on the farm until the end of the project. Otherwise we would have abandoned this kind of manager because it was an unproductive relationship. Trust between producer organizations and research institutions can be built up only with time (years working together) and experiencing both good (like Case 2) and bad outcomes (like this one). It is important to highlight both situations (good and bad) in terms of innovation, to make us more astute in choosing a cooperator to demonstrate new technology and to determine which characteristics of a farmer are important, to improve the effectiveness of the technology transfer process.

Conclusions

Extension theory suggests that demonstrations of research technology on commercial farms make the results more acceptable to other farmers than if it is implemented on a research facility. The control of forage allowance was possible without changing the average stocking rate of the farms by reallocation at paddock level and taking into account the animal energy demand. It is important to involve the manager and preferably surrounding farmers in discussions of the project from the outset, discussing the basis for the changes to be implemented. If possible, these same people should be involved in any measuring and monitoring that occurs during the exercise. In this way all involved are fully aware of the physical inputs needed to accurately estimate pasture availability and monitor animal performance to assess the benefits of the strategies implemented.

Despite our best intentions we were unsuccessful in convincing the cooperating farmers in Cases 3 and 4 to accept our recommendations, which highlights the difficulties involved in achieving technology adoption, even when the recommended methods for most effective technology transfer are utilized.

"Any change, even a change for the better, is always accompanied by drawbacks and discomfort": Arnold Bennett.

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Research Paper

Growth, yield and yield component attributes of narrow-leafed lupin (*Lupinus angustifolius* L.) varieties in the highlands of Ethiopia

Crecimiento, rendimiento y componentes del rendimiento de variedades de lupino dulce de hoja angosta (Lupinus angustifolius *L.) en las tierras altas de Etiopía*

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Abstract

An experiment was conducted to characterize the growth and yield performance of narrow-leafed sweet blue lupin varieties (*Lupinus angustifolius* L.) in northwestern Ethiopia. The experiment was laid out in a randomized complete block design with 4 replications and included 7 varieties (Bora, Probor, Sanabor, Vitabor, Haags blaue, Borlu and Boregine). Data on days to flowering and to maturity, flower color, plant height, numbers of leaflets, branches and pods per plant, pod length, number of seeds per pod, forage dry matter (DM) yield, grain yield and 1,000-seed weight were recorded. The results showed that plant height, number of branches per plant, forage DM yield, number of seeds per pod, grain yield and 1,000-seed weight varied significantly ($P<0.01$) among varieties. The highest forage DM yield at 50% flowering (2.67 t/ha), numbers of pods per plant (16.9) and of seeds per pod (4.15), grain yield (1,900 kg/ha) and 1,000-seed weight (121 g) were obtained from the Boregine variety. The tallest plants and greatest number of branches per plant were recorded from varieties Sanabor and Bora, respectively. Correlation analysis showed that the major factor affecting forage DM yield was plant height, while plant height, days to maturity and number of seeds per pod had the greatest influence on grain yield. The best performing variety was Boregine followed by Sanabor and Bora. These varieties seem promising for the development of sustainable forage production strategies with limited external inputs. However, future research should be conducted on the improvement of their agronomy and the possibility of their utilization as protein supplements using narrow-leafed sweet blue lupin forage or grain, as well as testing of promising varieties in diverse locations.

Keywords: Dry matter forage yield, grain yields, growth characteristics, legume crops.

Resumen

En la región noroeste de Etiopía se caracterizaron el crecimiento y rendimiento de siete variedades de lupino dulce de hoja angosta (*Lupinus angustifolius* L.): Bora, Probor, Sanabor, Vitabor, Haags blaue, Borlu y Boregine. El estudio se realizó en un diseño de bloque completos al azar y cuatro repeticiones. Se evaluaron el número de días a floración y madurez, el color de la flor, la altura de la planta, el número de folíolos, ramas y legumbres por planta, la longitud de la legumbre, el número de semillas por legumbre, la producción de materia seca (MS) forrajera, el rendimiento de grano y el peso de 1,000 semillas. Los resultados mostraron que la altura de la planta, el número de ramas por planta, la producción de MS forrajera, el número de semillas por legumbre, el rendimiento de grano y el peso de 1,000 semillas

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variaron significativamente (P<0.01) entre las variedades. La variedad Boregine presentó la mayor producción de MS (2.67 t/ha) al 50% de floración, el mayor número de legumbres por planta (16.9), el mayor número de semillas por legumbre (4.15), y el mayor rendimiento de grano (1,900 kg/ha) y peso de 1,000 semillas (121 g). Las plantas más altas y con el mayor número de ramas por planta se registraron para las variedades Sanabor y Bora, respectivamente. El análisis de correlación mostró que el factor principal que afectó el rendimiento de MS forrajera fue la altura de la planta, mientras que esta característica, los días hasta la madurez y el número de semillas por legumbre tuvieron la mayor influencia en el rendimiento de grano. La variedad de mejor desempeño fue Boregine seguida por Sanabor y Bora. Estas últimas variedades parecen promisorias para el desarrollo de estrategias de producción sostenible de forraje con insumos externos limitados. Sin embargo, se requieren estudios para mejorar la agronomía de estas variedades, evaluar la utilización del forraje o de los granos de lupino dulce como suplemento proteico en la alimentación animal y conducir pruebas multilocacionales de variedades promisorias.

Palabras clave: Características de crecimiento, leguminosas multipropósito, producción forrajera, rendimiento de grano.

Introduction

In Ethiopia, the livestock subsector makes significant contributions to the national income and the livelihoods of households (CSA 2016), contributing 15–17% of gross domestic product (GDP), 35–49% of agricultural GDP and 37–87% of household incomes (ILRI 2010; Behnke and Menagerie 2011). However, livestock's contribution is below potential because of inadequate nutrition, with the critical feed nutrient, crude protein (CP), of herbaceous plants declining during the dry season, leading to prolonged periods of under-nutrition of livestock (Yayneshet 2010). This shortage of CP could be mitigated through integration of multipurpose forage legume species such as lupin into feeding systems. Narrow-leafed sweet blue lupin (*Lupinus angustifolius* L.), hereafter referred to as narrow-leafed lupin, is adapted to low external input production systems, produces higher yields than local native lupins and is highly palatable to livestock, with Vitabor, Probor, Sanabor and Bora giving the highest grain yields. Vitabor and Sanabor are nationally registered varieties for use as multipurpose pulse crops in Ethiopia. These varieties have potential as both feed for livestock and food for humans and are suitable for intercropping with maize (Alemayehu et al. 2014; Yeheyis et al. 2015). While some research into adaptability and feeding value of narrow-leafed lupin has been conducted, no attempt has been made to characterize varieties. This study was conducted to characterize different varieties of narrow-leafed lupin based on growth characteristics and physical features.

Materials and Methods

Description of the study area

The study was conducted during June–November 2016 at Gondar Agricultural Research Station, which is located near Dabat town (13°05' N, 37°50' E; 2,740 masl) in the Dabat District of North Gondar Administrative Zone, Ethiopia (Figure 1). Rainfall and temperature data calculated for the research station are presented in Figure 2. The soil in the area is a well-draining Nitosol with low organic matter content (Table 1).

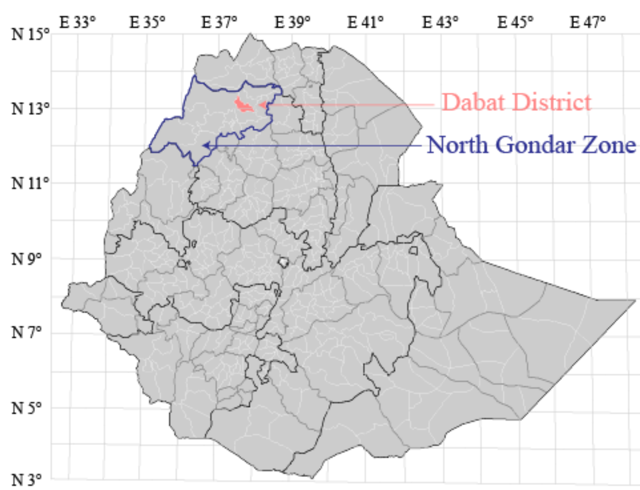

Figure 1. Map of Ethiopia showing the study area.

Table 1. Chemical properties of the soil in the experimental plots.

Parameter	Value
pH	6.13
Available P (ppm)	6.1
Organic matter (%)	1.42
CEC ($cmol_c$)	58.2
Exchangeable Ca ($cmol_c$)	41.5
Exchangeable Mg ($cmol_c$)	25.2
Exchangeable K ($cmol_c$)	2.21
Exchangeable Na ($cmol_c$)	0.31

Experimental design and treatments

The experimental design was a randomized complete block design with 4 replications. Plot size was 2.1 × 3.6 m (7.56 m^2), with 1 m between plots and replications. The land was plowed 3 times before planting to provide a fine seedbed. Seed was planted on 7 July 2016 in rows with 7 cm between plants within rows and a row spacing of 30 cm. Diammonium phosphate (DAP) fertilizer was applied at the rate of 100 kg/ha to provide 18 kg N and 22 kg P/ha at planting. Hand-weeding was conducted at the seedling stage and again prior to flowering.

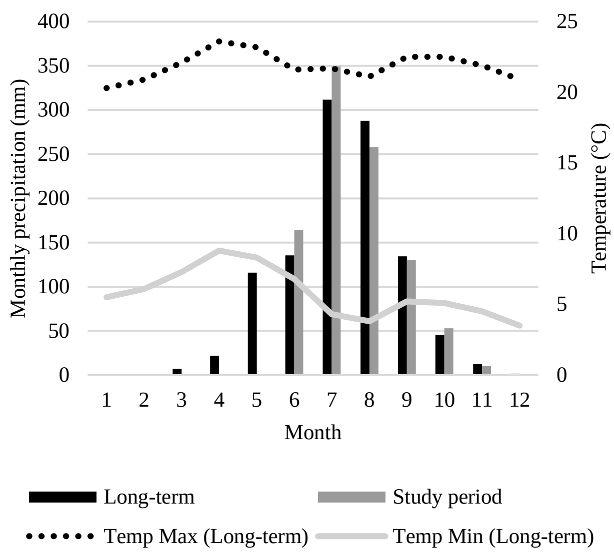

Figure 2. Climatic data calculated for the Gondar Agricultural Research Station, from Climate Hazard Group InfraRed Precipitation with Station (CHIRPS); long-term temperature data from WorldClim Version 2. Long-term data refer to 2007–2017. Data accessed through CCAFS GCM Downscaled Data Portal ccafs-climate.org/weather_stations.

Data collection

For sampling purposes half of each plot (subplot) was used to determine forage yield and the other half to determine grain production. Time from planting to flowering was assessed when 50% of plants in a plot were flowering and time to maturity when 90% of plants had reached maturity, i.e. set seed. Harvesting to determine forage biomass yield was performed at the 50% flowering stage from the middle 4 rows of half the subplots by cutting at ground level. Fresh biomass yield was recorded in the field and 500 g-samples were taken from each plot. Number of leaflets per plant was counted based on green leaves of 5 tagged plants at 50% flowering. These samples were separated manually into leaf and stem, before oven-drying at 65 °C for 72 hours until constant weight was obtained. Leaf:stem ratio was calculated. Plants on the remaining subplots were harvested at ground level at the optimum seed harvesting time (at 12.5% moisture) and seed was removed by threshing. Fresh weight of seed was determined and samples of seed were air-dried to constant weight.

Phenological and morphological parameters

Plant flower color was assessed based on close observation of flowers and recorded using a subjective scale from white to blue. Plant height was measured on 10 plants per plot, selected at random, from the soil surface to the tip of the longest leaflet at the forage harvesting stage. Number of leaflets and number of branches per plant were determined on 5 randomly tagged plants/plot at 50% flowering. Number of pods/plant, pod length and number of seeds/pod were measured on 5 randomly tagged plants/plot at maturity. To determine 1,000-seed weight, 1,000 seeds were taken randomly from the harvested seed lot for each plot and weighed.

Statistical analysis

The collected data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS (2016), Version 9.1.3. Mean separation was conducted using the least significant difference test (LSD), when the ANOVA showed significant difference among treatments. Simple pair-wise correlation and regression analyses were conducted using JMP 13 (SAS 2016) to show the relationship between growth, yield and yield components. The statistical model used for data analysis was:

$$Y_{ij} = \mu + t_i + b_j + e_{ij}$$

where: Yij = the response variable (the observation in jth block and ith treatment); μ = the overall mean; ti = the treatment effect; bj = the block effect; and (gradient) eij = the random error.

Results

Days to flowering and maturity and flower color

Variety significantly (P<0.01) influenced both days to flowering and days to maturity (Table 2). The longest time to flowering was for Bora (87.5 days) and the shortest for Haags blaue (74.5 days), while corresponding times to maturity were 147 and 134 days, respectively. Haags blaue variety was seriously affected by lack of moisture at the flowering stage and aborted flowers before it reached maturity. Varieties Probor, Vitabor, Haags blaue and Borlu showed blue flower color, while flowers of Bora and Sanabor were bluish white; only those of Boregine were white.

Plant height

Plant height was significantly (P<0.05) affected by variety with tallest plants for Sanabor (67.6 cm) and shortest for Vitabor (53.8 cm; Table 2).

Number of branches and leaflets per plant

Lupin has an indeterminate growth habit and can continue to initiate new lateral branches with flower spikes after it reaches the reproductive phase. The highest number of branches was observed for Bora (22) and lowest for Haags blaue (16) (P<0.01). Number of leaflets per plant followed a similar pattern with highest number for Bora (173) and lowest for Haags blaue (78) (P<0.01) (Table 2).

Forage dry matter yield

Forage dry matter yield at flowering varied significantly (P<0.01) among varieties with yields varying from 2.69 t/ha for Bora to 1.23 t/ha for Vitabor. While leaf:stem ratio varied from 2.40 for Bora to 1.52 for Boregine, differences were not significant (P>0.05) (Table 2).

Number of pods per plant, seeds per pod and seed (grain) yield

The number of pods per plant was not significantly (P>0.05) affected by variety with an overall mean of 11.0 and a range of 8.1 to 16.9 pods/plant (Table 3). Similarly, pod length was not affected by variety. However, number of seeds per pod was significantly (P<0.001) affected by variety ranging from 3.18 to 4.15.

Grain yields varied widely between varieties (P<0.01), ranging from 1,901 kg/ha for Boregine to 786 kg/ha for Haags blaue (Table 3). Thousand-seed weight also varied significantly between varieties from 125 g for Sanabor to 91 g for Borlu.

Correlation between plant parameters and grain yield

Almost all agronomic attributes were strongly correlated with grain yield. Plant height (r = 0.59**), days to flowering (r = 0.51**), days to maturity (r = 0.56**), number of seeds per pod (r = 0.92**) and number of branches per plant (r = 0.52**) were positively related to grain yield (Table 4).

Table 2. Effects of variety on days to flowering (FD), plant height (PH), branches per plant (NB), leaflets per plant (NL), leaf:stem ratio (L:S), days to maturity (MD) and forage dry matter yield (DMY) of 7 narrow-leafed lupin varieties.

Variety	FD (no.)	PH (cm)	NB (no.)	NL (no.)	L:S	MD (no.)	DMY (t/ha)
Bora	87.5a[1]	64.3abc	22.0a	173a	2.40	147a[1]	2.69a
Probor	81.5bc	60.1bcd	19.5d	129c	2.25	141c	1.95b
Sanabor	87.0a	67.6a	21.2bc	155ab	2.08	146ab	2.49ab
Vitabor	78.5c	53.8d	22.0ab	110cd	2.05	138d	1.23c
Haags blaue	74.5d	59.4cd	16.1e	78d	1.99	134e	2.10b
Borlu	81.3bc	64.2abc	18.8d	107d	1.82	141c	2.07b
Boregine	84.5ba	66.3ab	20.7c	151a	1.52	144b	2.67a
Overall mean	82.1	62.2	20.0	129	1.87	142	2.17
CV (%)	3.26	6.96	2.61	10.56	12.87	1.98	17.13
s.e.	2.68	4.33	0.52	13.62	0.24	1.40	0.37
Significance	***	*	***	***	ns	***	***

[1]Values within columns followed by different letters are significantly different.

Table 3. Effects of variety on number of pods per plant (NP), pod length (PL), number of seeds per pod (NS), grain yield (GY) and 1,000-seed weight (TSW) of 7 narrow-leafed lupin varieties.

Variety	NP (no.)	PL (cm)	NS (no.)	GY (kg/ha)	TSW (g)
Bora	11.9	3.73	3.94ab[1]	1,715ab	118a
Probor	12.2	3.35	3.18b	1,129c	98b
Sanabor	10.2	3.75	4.13ab	1,794ab	125a
Vitabor	8.1	2.65	3.70ab	1,257bc	96b
Haags blaue	12.7	3.68	3.18b	786bc	102b
Borlu	8.9	3.5	3.38ab	1,233bc	91b
Boregine	16.9	3.78	4.15a	1,901a	121a
Overall mean	11.0	3.49	3.67	1,402	107
s.e.	3.18	0.66	0.37	39.86	9.57
CV (%)	28.97	18.90	10.21	27.95	8.93
Significance	ns	ns	***	**	***

[1]Values within columns followed by different letters differ significantly (P<0.05).

Table 4. Correlations between plant parameters, yield and yield components of 7 narrow-leafed lupin varieties.

	PH[1]	FD	MD	NB	DMY	NP	NS	GY	TSW	L:S	PL	NL
PH	1											
FD	0.45*	1										
MD	0.46*	0.97**	1									
NB	0.15	0.62**	0.65**	1								
DMY	0.75**	0.35	0.42*	0.103	1							
NP	0.39*	-0.11	-0.07	-0.10	0.51**	1						
NS	0.51**	0.45*	0.49**	0.55**	0.41*	0.31	1					
GY	0.59**	0.51**	0.56**	0.52**	0.46*	0.43*	0.92**	1				
TSW	0.58**	0.45*	0.48*	0.36	0.67**	0.57**	0.74**	0.71**	1			
L:S	0.41*	0.66**	0.64**	0.62**	0.20	-0.12	0.47*	0.40*	0.26	1		
PL	0.50**	0.14	0.16	-0.11	0.56**	0.55	0.40*	0.48*	0.64**	-0.05	1	
NL	0.51**	0.78**	0.82**	0.74**	0.53**	0.17	0.53**	0.57**	0.56**	0.68**	0.19	1

[1]PH = plant height; FD = days to flowering; MD = days to maturity; NB = number of branches per plant; DMY = forage dry matter yield; NP = number of pods per plant; NS = number of seeds per pod; GY = seed yield; TSW = 1,000-seed weight; L:S = leaf:stem ratio; PL = pod length; NL = number of leaflets per plant.

Discussion

This study has provided useful information on the possible yields of forage and seed from narrow-leafed lupin varieties in Ethiopia. The variation in these parameters among different varieties suggests that there is room for selection if the aim is to establish breeding programs to improve yields.

Days to flowering

Mean time to flowering for Sanabor of 87 days was similar to that reported by Herbert (1977), who showed that flowering began at about 84 days after sowing and most plants were flowering by 87 days.

Flower color

We observed that Boregine was the only variety that had white flowers, which was in agreement with Tarekegn (2016), who indicated that Boregine displayed different flower colors including white. Lupin flowers do not contain nectar, but insects are attracted by their bright color and presence of pollen (Kurlovich et al. 2011). Klamroth et al. (2011) suggest that different flower colors in lupins might improve agronomic characters such as seed yield by attracting pollinating insects.

Days to maturity

As might be expected, varieties that flowered early also reached maturity before other varieties. Berger et al. (2011) indicated that delayed phenology has a huge effect on yield. Based on a study with white lupins, where varieties had set seed by 4.5 months, Azeze (2016) suggested that, as compared with other pulse crops, white lupin must remain in the field for 7–8 months until the plant fully matures. Mulugeta et al. (2015) suggested that the presence of highly significant variation in maturity between Sanabor and the Ethiopian landraces indicates the possibility of using Sanabor as a source of genes to advance flowering in late-maturing Ethiopian landraces.

Plant height

The difference in plant height between varieties was in agreement with Tarekegn (2016), who stated that the tallest plants were recorded in Bora and Sanabor and the shortest plants in Vitabor. We recorded an overall mean plant height of narrow-leafed lupin varieties of 62 cm (range 54–68 cm) as opposed to the mean height of 82 cm reported for the same varieties by Rudloff (2011). This discrepancy might be due to environmental variations of experimental areas. However, Edwards et al. (2011) reported that plant height of other narrow-leafed lupin varieties varied from 20 to 100 cm.

Number of branches per plant

Growth habit of lupin is characterized by a high number of branches per plant when plants have access to adequate moisture, nutrients and sunlight (Edwards et al. 2011). While we recorded an average of 20 branches per plant, Rudloff (2011) reported for Boruta lines (also *L. angustifolius*) that the maximum number of branches per plant was 7. This might be a function of agro-ecological differences and management variations. It was interesting that there was no significant correlation between plant height and number of branches per plant. In contrast with this study, Klamroth et al. (2011) found that the strongest vigor was observed in

Boregine, followed by Haags blaue, with Probor showing only marginal strength.

Seeding

The mean grain yields of all varieties in this study were below expected levels which might be due to the poor soil in the study area. Root elongation of narrow-leafed lupin slows when the pH ($CaCl_2$) increases from 5.5 to 6.0, and at a pH greater than 6.0 the root surface can be damaged as surface cells are peeled off and root hair formation slows (Edwards et al. 2011). In this experiment the pH of the soil was 6.3. In addition, the flowering process of lupin is affected by high temperatures which cause blasting of flowers and a subsequent grain yield reduction (Klamroth et al. 2011); blasting of flowers was also observed in this experiment.

Late-flowering varieties such as Boregine and Sanabor had the highest grain yields as they had the highest numbers of pods per plant, seeds per pod and 1,000-seed weight compared with the other genotypes. Late flowering and hence late seed set could have negative consequences in some circumstances as the grain-filling period may coincide with increasing temperatures and decreasing soil water availability with associated decrease in availability of nutrients, leading to terminal drought and yield reductions (Thornton et al. 2014). Lupin appears to rely on current photosynthesis to fill grain, as opposed to e.g. wheat, which remobilizes stored carbon fixed earlier in the season. This phenomenon may account for high pod abortion, poor yield stability and low harvest index (Sandaña et al. 2009; Edwards et al. 2011).

The ranking of varieties in terms of grain yield was at variance with the previous report of Yeheyis et al. (2012), who reported yield rankings in descending order: Vitabor, Sanabor, Probor and Bora, in northwestern Ethiopia. These differences could be due to variation in agro-ecologies among the study areas. Tarekegn (2016) also found that Sanabor gave higher grain yield than other tested narrow-leafed lupin genotypes, while Yeheyis et al. (2015) indicated that Boregine and Sanabor were the best-performing varieties among the present tested genotypes. While Klamroth et al. (2011) showed highest yields for Boregine, Mekonnen et al. (2016) showed that varieties Sanabor, Vitabor, Bora and Probor gave comparable grain yields and 1,000-seed weights when intercropped with maize.

Kurlovich et al. (2011) also reported no significant difference in number of pods per plant among narrow-leafed lupin varieties. Number of seeds per pod is a major yield component and breeding target in lupins that shows great variation and is invaluable for genetic improvement (Yang et al. 2016). Our observations indicated that number of seeds per pod had a much greater influence on grain yields than number of pods per plant. Pods can produce 3 to 6 seeds, depending on the location on the plant, with pods produced on higher branches containing fewer seeds (Edwards et al. 2011). Though the overall mean number of seeds per pod obtained from this experiment was less than that reported by Rudloff (2011), significant differences ($P<0.001$) in number of seeds per pod were exhibited among varieties as shown by Yang et al. (2016). Differences were not surprising as this is a highly complex trait determined by the number of ovules per ovary, the proportion of fertile ovules (ovule fertility), the proportion of ovules fertilized and the proportion of fertilized ovules that develop into seeds. The proportion of ovules fertilized is determined by the fertilization process, which is dependent on many factors such as pollen sterility, the amount of pollen deposited on the stigma, pollen grain germination (Schreiber and Dresselhaus 2003) and pollination conditions (Brown et al. 1990). Among the tested varieties Klamroth et al. (2011) discovered that flower number and pod set in Boregine were lowest, resulting in low seed production. In another experiment conducted by Mekonnen et al. (2016) numbers of narrow-leafed lupin seeds per pod and pods per plant did not show significant difference among treatments when they were intercropped with maize.

Thousand-seed weight

The finding that Sanabor, Boregine and Bora gave the highest 1,000-seed weights (125, 121 and 118 g, respectively) was in parallel with the finding of Stoddard and Lizarazo (2011). These high 1,000-seed weights could be attributed to large accumulation of assimilates due to a long maturity period. The overall mean 1,000-seed weight (107 g) obtained from this experiment was much lower than the 130 and 143 g reported by Edwards et al. (2011) and Rudloff (2011), respectively, the differences being probably due environmental conditions.

Forage dry matter yield

The pattern of forage DM yield, i.e. highest in Boregine and lowest in Haags blaue, was in agreement with findings of Yeheyis et al. (2011). On the other hand, other studies, e.g. by Tarekegn (2016), showed no difference in mean DM yields per season of narrow-leafed varieties. We also found that the longer plants took to mature the higher were the forage and seed yields as there was sufficient time to accumulate biomass and set greater amounts of seed. Weerakoon and Somaratne (2013) showed that variety had a statistically significant effect on biomass yield. While we did not analyze forage or seed for crude protein concentration, other workers have shown that forage of narrow-leafed lupin contains CP concentration of 15.8–30% at maturity, which makes it suitable for animal feeding (Yeheyis 2012). A 70-day study conducted with Washera sheep using narrow-leafed lupin grain as a supplement at 290 g/head/day showed that the animals gained 74 g/head/day (Yeheyis et al. 2012). The same study showed that cv. Sanabor has potential to be a suitable substitute for commercial concentrate feed supplement in Ethiopia and concludes that lupin seed is a highly nutritious product which can be used as a protein and energy supplement for livestock during dry times.

Conclusions and Recommendations

This study has shown that the narrow-leafed lupin varieties Boregine, Sanabor and Bora appear better suited to the study area than the other varieties tested because of better forage DM and grain yields. These varieties seem promising for the development of sustainable forage production systems with a limited use of external inputs as they are common in Ethiopian smallholder agriculture. However, performance over a range of seasons and locations is necessary to confirm these initial findings. Future research on narrow-leafed lupin should be directed at a more detailed study of its grain yield components, quality of both forage and seed, the improvement of its agronomy and the possibility of its utilization as forage or green manure crop, as well as to the testing of promising genotypes in diverse locations.

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(Note of the editors: All hyperlinks were verified 17 January 2019.)

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